Bulletin No. 20 from the Department of Surgery,
University of Lund, S - 221 85 LUND,
1980

STIMULATION OF GASTRIC ACID SECRETION

Vagus - gastrin - histamine interactions in the rat

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by

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Lund 1980

The present thesis is based on the following papers, which will be referred to in the text by their roman numerals.

I. Activation of histidine decarboxylase by H_2-receptor blockade (in collaboration with R. Håkanson, G. Liedberg, J.F. Rehfeld and F. Stadil), Br. J. Pharmac. (1975), 53, 127-130.

II. Gastrin: Obligatory intermediate in the post-prandial mobilization of gastric histamine in the rat (in collaboration with R. Håkanson, G. Liedberg, H. A. El Munshid and J.F. Rehfeld), Experientia (1977), 33, 1541 - 1542.

III. Gastrin cell proliferation after chronic stimulation: Effect of vagal denervation or gastric surgery in the rat (in collaboration with J. Alumets, H. A. El Munshid, R. Håkanson, G. Liedberg, J. Oscarson, J. F. Rehfeld, F. Sundler and S. Vallgren), J. Physiol. (1980), 298, 557-569.

IV. Effects of insulin on the serum gastrin concentration, gastric acid secretion and histamine mobilization in the rat (in collaboration with R. Håkanson, G. Liedberg, I. Lundquist, J. F. Rehfeld and F. Sundler). Submitted for publication.

V. Intraluminal factors in the activation of rat stomach histidine decarboxylase (in collaboration with M. Ekelund, R. Håkanson, G. Liedberg and S. Vallgren). Submitted for publication.

VI. Mechanism of gastric acid secretion after pylorus and oesophagus ligation in the rat (in collaboration with R. Håkanson, G. Liedberg, F. Sundler and S. Vallgren). J. Physiol. (in press)

Acid in the stomach has always received more attention than it deserves.

Horace W. Davenport, 1978

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1. Background

In his pioneering work, William F. Beaumont demonstrated that the stomach secretes a clear fluid and that the volume of this gastric secretion can be influenced by external stimuli (Beaumont 1833). On samples provided by Beaumont, Berzelius showed that the gastric juice contained large amounts of hydrochloric acid (see Davenport 1978).

At the turn of the century Pavlov demonstrated that the secretory response to sham feeding could be abolished by vagotomy indicating that the vagus nerves mediate excitatory stimuli to the gastric glands (Pavlov 1898).

The next step was taken by Edkins (1906) who demonstrated the secretory effect of an extract of pyloric mucosa. He named the new factor gastrin.

The secretory potential of histamine was recognized a few years later (Abel & Kubota, 1919) and for a long time it was argued that the secretory effect of Edkin's extract was due to contaminating histamine (Sachs et al., 1932). That controversy was put to an end by the demonstration that a histamine-free extract of antral mucosa stimulated gastric acid secretion (Komarow 1938). Four years later Uvnäs demonstrated that vagal excitation affected gastrin release as well as acid secretion (Uvnäs 1942). He also provided evidence for a synergistic interaction between gastrin and vagal activity on the parietal cells.

The nature and identity of gastrin was revealed in 1964 when the hormone was isolated and chemically defined (Gregory and Tracy, 1964).

In 1972 a series of specific histamine H_2-receptor antagonists was introduced (Black et al., 1972). They were found potent in inhibiting basal as well as stimulated gastric acid secretion, suggesting a physiological role for histamine.

2. The Classical Concept

Traditionally, stimulation of gastric acid secretion has been thought to occur in three phases. It is important to note that their names merely indicate the proposed site of origin of the stimulus. Each phase may act through nervous as well as hormonal pathways.

A. Cephalic phase

The cephalic phase of gastric acid secretion is initiated in the central nervous system and evoked by various stimuli, such as the sight, smell, taste and expectation of food (Pavlov 1898). Cerebral intracellular glucopenia, induced by the injection of insulin or 2-deoxy-D-glucose, stimulates gastric acid secretion through a vagal pathway (Brooks, 1965; Hirschowitz & Sachs, 1965). The enhanced vagal impulse flow results in a direct stimulatory effect on the parietal cells (Pevsner & Grossman, 1955) and on the release of gastrin (Pe Thein & Schofield, 1959). The concept that insulin-induced gastrin release is solely mediated by the vagus has recently been challenged (Brandsborg et al., 1975; Christensen & Stadil, 1976).

B. Gastric phase

The gastric phase is initiated by food entering the stomach. Mechanical stimulation e.g. in the form of distension of the gastric fundus causes an increase in acid secretion (Richardson el al., 1976; Grötzinger et al., 1977). Chemical stimulation (amino acids, peptides, elevated pH) of the antral mucosa leads to gastrin release (Elwin & Uvnäs, 1966) and

to an enhanced rate of acid secretion (Richardson et al., 1976). There is also some evidence for a pyloro-oxyntic reflex elicited by distension (Debas et al., 1974; Bergegårdh & Olbe, 1975).

C. Intestinal phase

The intestinal phase of gastric acid secretion is initiated when food enters the small intestine. A humoral agent is then released from the jejunum (Orloff et al., 1970). It augments both gastrin- and histamine-induced acid secretion (Debas et al., 1975) and is inactivated in the liver (Orloff et al., 1969). Also a nervous reflex may be involved in the acid response to food in the small intestine (Buxton et al., 1972).

It is important to note that although the three phases occur sequentially they overlap grossly. Each phase probably acts through both neural and hormonal pathways and there is considerable interaction between them. The classical three phases have been defined according to the site of origin of the stimulus. An alternative way of studying gastric secretion would be to examine separately the mechanisms stimulating the cell types believed to be involved in the chain of events leading to gastric acid secretion.

3. Gastric Mucosal Cell Types Studied

A. The gastrin cell

In the adult rat gastrin-containing cells (G cells) are found almost exclusively in the mucosa of the gastric antrum (Larsson et al., 1974). The G cell has a triangular shape with its apex in contact with the

gastric lumen (McGuigan, 1968). Microvilli protrude from its apical surface. The microvilli are thought to carry receptors enabling the cell to respond to stimuli from the gastric lumen. The nucleus is located in the basal third of the cell. Granules believed to contain the hormone surround the nucleus and are predominantly located basally in the cell. In the electron microscope these granules display a varying electron density. The reasons for this variability in electron density are not clear.

Gastrin is released from the gastrin cell by a mechanism not yet fully understood. Release by exocytosis is supported by indirect evidence (Sato, 1978), although no granules opening themselves onto the surface of the gastrin cell ("omega figures") have been identified. Gastrin is claimed to be released not only into the circulation but also into the lumen of the stomach (Andersson & Nilsson, 1974; Uvnäs-Wallensten, 1977, 1978).

The rate of gastrin release is regulated by excitatory and inhibitory stimuli. The vagus nerve seems to contain stimulatory as well as inhibitory fibres (Schafmayer et al., 1978). Chemical stimulation of the gastrin cell can be exerted from the lumen of the stomach. It has been proposed that receptors on the microvilli mediate this effect. Acetylcholine (Grossman et al., 1948), certain amino acids (Thompson, 1969), calcium ions (Levant et al., 1973) as well as a high antral pH (Walsh et al., 1975) enhance the rate of gastrin release, whereas a low antral pH inhibits it (Redford & Schofield, 1965; Becker et al., 1973).

B. The histamine-storing endocrine cells

In most mammals, including man, gastric mucosal histamine is stored in mast cells in the lamina propria. In the rat, however, the bulk of gastric histamine is in enterochromaffin-like cells which occur at the bottom of the oxyntic glands (Håkanson & Owman, 1967; Thunberg, 1967). Ultrastructurally, the histamine-storing cells comprise two main cell types, the ECL cell and the A-like cell. The two cell types are distinguishable by their cytoplasmic granules (Håkanson et al., 1976a).

The activity of these cells is reflected in the activity of the histamine-forming enzyme histidine decarboxylase (see Håkanson et al., 1976a,b). The activity of the enzyme seems to be under the exclusive control of gastrin (Håkanson et al., 1974, 1976b) although claims have been made that a direct vagal innervation may contribute (Kahlson et al., 1976; Rosengren & Svensson, 1969; Lundell, 1976). Agents or treatment that release gastrin as well as the injection of gastrin itself cause a rapid initial fall in the mucosal content of histamine and an increased activity of histidine decarboxylase, indicating mobilization of histamine and a general activation of the histamine cells (Håkanson et al., 1974, 1977). In the absence of endogenous gastrin, only the injection of gastrin or gastrin-like agents seems to be capable of activating the enzyme (Håkanson & Liedberg, 1970, 1971a). Thus, gastrin seems to regulate the functional activity of the enterochromaffin-like cells. In addition gastrin exerts a trophic influence on these cells (Håkanson et al., 1976a; Alumets et al., 1979b).

C. The parietal cell

Parietal (or oxyntic) cells are predominantly found in the middle third of the oxyntic glands. The cells are round or ovoid and lack secretory granules but have a network of canaliculi. In the presence of stimulants of gastric acid secretion the parietal cells undergo characteristic morphological transformation resulting in an increased secretory surface (Helander & Hirschowitz, 1972; Forte et al., 1975, 1977) and secretion of hydrochloric acid.

Considerable controversy has existed regarding the mechanism by which the parietal cell is activated. Three endogenous compounds are potent in stimulating acid secretion, viz. gastrin, histamine and acetylcholine. According to one theory all secretagogues act through the release of histamine which is considered to be the final stimulus for the parietal cells (McIntosh, 1938; Kahlson et al., 1964; Code, 1965; Kahlson et al., 1973). The cells are seen as driven by a histamine-producing power plant. The rate a which gastric histamine is produced and released under physiological conditions is thought to be controlled by gastrin and by the vagal activity. The more histamine that is generated and mobilized, the more acid is secreted by the parietal cells.

According to this concept the activity of the parietal cells should reflect the activity of the histamine-producing cell system. There are several instances where such a correlation does not exist. Thus, in vagally denervated rats the rate of histamine formation is high whereas the rate of acid secretion is very low. The administration of atropine

or H_2-receptor blockers activates the histamine-forming enzyme in the gastric mucosa but inhibits the acid secretion. Further, both secretin and somatostatin inhibit the acid response to gastrin (Tumpson & Johnson, 1969; Konturek 1976; El Munshid et al., 1980b). According to the above concept this effect should be exerted either on the histamine-producing cells (blockade of gastrin-induced activation) or on the parietal cells (blockade of histamine-induced secretion). Previous as well as more recent results suggest that neither secretin nor somatostatin interferes with the response of the histamine-producing cells to gastrin (Caren et al., 1969; Alumets et al., 1979; El Munshid et al., 1980b). Also, neither secretin nor somatostatin seems to inhibit the response of the parietal cells to exogenous histamine (Johnson & Tumpson, 1970; Konturek, 1976; El Munshid et al., 1980b). Together these observations favour the view that somatostatin and secretin act by blocking the effect of gastrin at the parietal cell level and not at the histamine cell level. The situation induced by nephrectomy is somewhat similar. Nephrectomy in the rat causes marked hypergastrinemia and activation of the histamine-forming endocrine cells but the rate of basal acid secretion remains unchanged. Under these conditions exogenous histamine retains its capacity to stimulate acid secretion, whereas the response of the parietal cells to gastrin is inhibited (El Munshid et al., 1976, 1980a). There are still other arguments against the concept that stimulation of acid secretion is dependent on the local formation and release of histamine.

Hexamethonium, for instance, blocks histamine-induced acid secretion but not that induced by pentagastrin (Håkanson & Liedberg, 1971b). The calculated maximal acid response to gastrin is significantly greater than that to histamine (Johnson, 1971). Further, both in the rat and the dog potentiation occurs between gastrin and histamine. Potentiation is believed to require two receptor sites; hence it is assumed that no two agents showing potentiation act through one receptor (Johnson, 1971). On the whole these observations do not favour the view that histamine is the final common mediator of gastric acid secretion. Nonetheless, the injection of specific histamine H_2-receptor antagonists (Black et al., 1972) inhibits basal as well as stimulated acid secretion in all species studied. This observation speaks in favour of a physiological role for histamine in the control of gastric acid secretion.

A possible explanation for these apparently contradictory findings is offered by the theory of "permissive action" (Grossman, 1975). According to this theory the parietal cell has separate but interdependent receptors for histamine, gastrin and acetylcholine. Blocking the histamine receptor alters the responsiveness to gastrin and to cholinergic agents. Similarly, blocking the cholinergic receptor with atropine alters the responsiveness to histamine and to gastrin. This theory presupposes that histamine and acetylcholine reach the parietal cells from some adjacent tissue source. Whether blockade of the gastrin receptor alters the responsiveness to histamine and acetylcholine is unknown.

4. Aims of the Present Investigation

The aims of the present investigation were to study mechanisms in the gastric mucosa associated with gastric acid secretion. Specific questions asked were:

1/ How does the intragastric milieu affect the gastrin cells in the pyloric glands and the histamine-storing cells in the oxyntic glands?

2/ What is the role of the vagus in the acid secretory response to pyloric ligation?

3/ What is the mechanism behind the acid secretory effect of insulin?

5. Materials and Methods

The details of the laboratory techniques are given in the respective papers. Only a brief summary will be given here.

A. Animals

Adult male rats of the Wistar or Sprague-Dawley strains were used. The rats were fed a standard diet of food pellets and tap water. Prior to experiments the rats were kept in individual cages with wire mesh bottoms to prevent coprophagia. The fasting period was 48 hours prior to studies on histidine decarboxylase and 24 hours prior to studies on acid output.

B. Surgical procedures

Rats were operated upon with clean but not sterile instruments unter light diethyl ether or chloralose anesthesia (as specified). To prevent fluid loss during experiments all rats received 10 ml of 0,9 % saline subcutaneously. The various experimental models are schematically presented in Fig. 1.

C. Collection and titration of gastric juice

In pylorus-ligated animals (with or without oesophagus ligature) the abdomen was closed and the rats allowed to recover from the anesthesia. At the end of the test period the animals were again anesthetized, the abdomen opened and the oesophagus clamped when necessary. The entire stomach was then removed, opened along the greater curvature

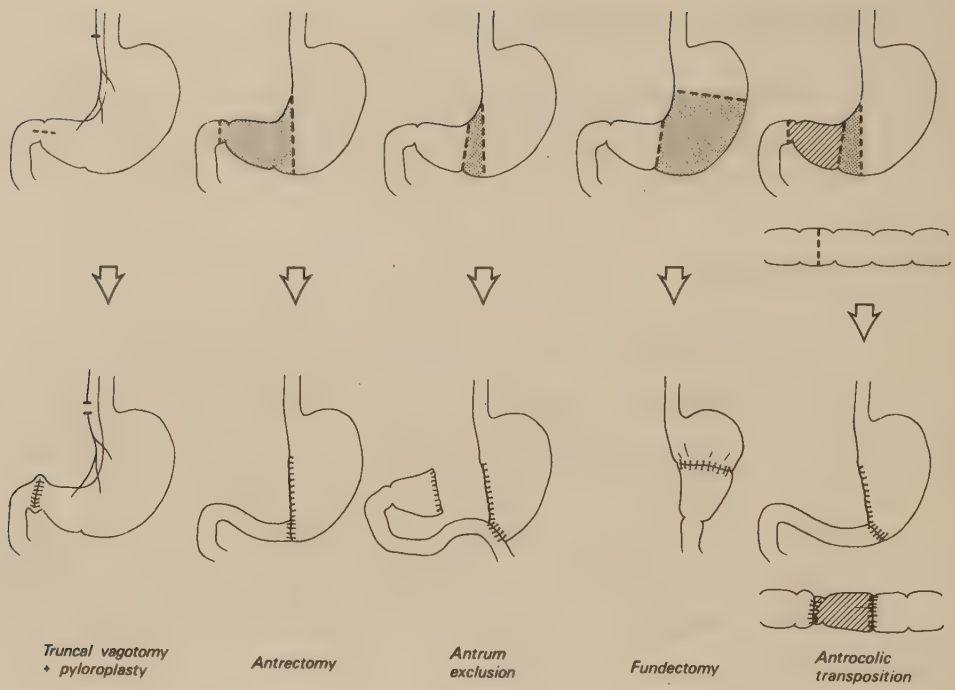

Fig.1. Schematic presentation of the operative procedures employed. A 1-2 mm resection of the antro-fundic junction was performed in antrum exclusion and in antro-colic transposition to ensure that no parietal cells remained in the antral portion.

and its contents drained into a test tube. The stomach was rinsed with 1 ml of re-destilled water. The test tube was then centrifuged and the supernatant titrated with 0.02 M NaOH using phenolphthalein as an indicator. The results were expressed as micro-equivalents H^+ per four hours.

Another technique for the collection of gastric juice employed chronic gastric fistulas inplanted into the rumen of the stomach (Bel et al., 1966). Rats were allowed to recover from this operation for at least three weeks, during which time they were thoroughly acquainted with the restraining

cages and the conditions of the experiments. After 24 hours of fasting the rat was lightly anesthetized with ether, the gastric fistula opened and the stomach rinsed with warm saline (+37°C) until the return was clear. The animals were then put into the restraining cages with the fistula open. After one hour of free flow, two one hour portions of basal acid secretion were collected whereafter the stimulating agent was administered. Four further one-hour portions were collected. The portions were centrifuged and titrated in the manner described above. The results were expressed as microequivalents H^+ per hour.

D. Gastric perfusion

After 48 hours of fasting the rats were anesthetized with chloralose (1-1,5 ml i.p., 5 % solution) and the abdomen opened through a midline incision. The inflow catheter was inserted through the rumen and secured with a purse string suture. During testing anesthesia was maintained by adding chloralose to the abdominal cavity. During experiments with low perfusion rates (1.2 ml/h) no active drainage of the stomach was needed to prevent gastric distension. During experiments with a high rate of perfusion (16 ml/h) drainage was effected by severing the duodenum and bringing out the proximal end through the midline incision.

E. Serum sampling and gastrin determination

Blood was drawn from the abdominal aorta, allowed to clot, and then centrifuged. Serum was collected, lyophilized, and stored in the deep freeze until

gastrin analysis. Gastrin was determined using a radioimmunoassay technique (Stadil & Rehfeld, 1971). The specificity of the assay and its application to rat serum has been described previously (Håkanson et al., 1974).

The mucosa was scraped off the pyloric gland area with a scalpel, weighed immediately and homogenized in boiling, re-distilled water for 20 minutes. The samples were then centrifuged and the supernatant stored in the deep freeze until analysed for gastrin by radioimmunoassay as described above.

F. Determination of mucosal histamine content and histidine decarboxylase activity

Histamine was determined fluorometrically in deproteinized extracts of gastric mucosal homogenates (Håkanson, Rönnberg, Sjölund, 1972; Håkanson & Rönnberg, 1974). For determination of the gastric histidine decarboxylase activity the mucosa of the oxyntic gland area was homogenized in 0.1 M phosphate buffer (pH 6.9 - 7.0). Aliquots of the homogenates were incubated with ^{14}C-carboxy-labelled histidine. The amount of $^{14}CO_2$ produced was measured by liquid scintillation counting (for details see Håkanson et al., 1974, 1977).

G. Drugs and chemicals

Synthetic human gastrin-I (1-17) (15-leu) was purchased from Fluka AG, Basle, Switzerland. Commercially available pentagastrin (Peptavlon ^{R} ; ICI), insulin (Vitrum, Sweden), metiamide (Smith, Kline & French), chlorisondamine (Ciba-Geigy), atropine (ACO, Sweden) were used.

6. Results and Comments

A. Activation of the gastrin cell

Feeding causes release of gastrin into the circulation (see for instance II). This release is probably due to vagal stimulation and to local effects in the antrum caused by food constituents and by buffering of the antral contents. Preventing acid from gaining access to the antrum (by antrum exclusion or fundectomy) or disminishing the acid output (vagotomy, fundectomy, H_2-receptor blockade), thereby raising the antral pH, increased the serum gastrin concentration (I, II, III). A contribution of the vagi to the pH-stimulated gastrin release process is suggested by the fact that rats with fundectomy + vagotomy had a lower serum gastrin concentration than rats with fundectomy alone (III). Insulin-induced hypoglycemia, which is thought to stimulate vagal activity, also caused gastrin release (IV). However, the effects of insulin cannot be equated with those of vagal excitation, since the combined injection of insulin + glucose in doses giving hyperglycemia or euglycemia evoked the same gastrin response as insuline alone. Also, insulin raised the serum gastrin concentration in vagally denervated rats. Therefore, insulin seems to release gastrin at least partly by mechanisms unrelated to vagal excition (IV).

Long term stimulation of the gastrin cells might be expected to result in their proliferation. This assumption was confirmed (III). The combination of elevated antral pH and the passage of food proved to be mandatory for gastrin cell proliferation (III). The gastrin cell density was higher after fundectomy

(when the passage of food stimulates the antrum) than after antrum exclusion; both these preparations preclude acid from reaching the antrum. The quality of the material passing the antrum seems to be important, since transposing the antrum to the colon failed to elicit gastrin cell proliferation.

B. Activation of the histamine-storing endocrine cells

The histamine-storing endocrine cells in the rat gastric mucosa seem to be under the control of endogenous gastrin. Several observations support this view (for references see Håkanson et al., 1976 b). Exogenous gastrin or pentagastrin, given by the subcutaneous route, effectively activates the histamine-forming enzyme, histidine decarboxylase, and mobilizes histamine (reflected in reduced concentrations of mucosal histamine)(Kahlson et al., 1964, 1973; Håkanson et al., 1976 b). In addition, the enzyme was activated and mucosal histamine was mobilized by raising the serum gastrin concentration, for instance by feeding (II) or by H_2-receptor blockade (I). Several other treatments that raise the serum gastrin concentration also raise the gastric histidine decarboxylase activity (for references see Håkanson et al., 1976b).

While systemically administered gastrin readily activated gastric histidine decarboxylase (I, II, V), this effect could not be reproduced by gastrin administered by gastric perfusion (V).

It has been claimed that vagal excitation may activate the histamine-storing endocrine cells independently of gastrin (Kahlson et al., 1973). This hypothesis was examined in papers II and IV. The effect of insulin hypoglycemia is thought to be mediated by

vagal excitation. In doses from 0.3 to 10.0 U/kg insulin activated gastric histidine decarboxylase and, though less pronounced, caused a fall in mucosal histamine content. Low doses of insulin activated histidine decarboxylase despite the fact that there was no increase in the serum gastrin concentration, possibly due to a direct vagal involvement in the enzyme activation. Alternatively, the enzyme activation following low doses of insulin may be explained by an enhanced sensitivity to gastrin as a result of insulin pretreatment. Such an enhancement was suggested from our results (IV). Vagal excitation evoked by feeding did not cause mobilization of mucosal histamine or activation of histidine decarboxylase in antrectomized rats (II). On the whole, therefore, it seems more likely that vagal excitation activates the histamine-storing endocine cells through the mediation of gastrin.

When hypoglycemia was prevented by giving glucose together with insulin, the serum concentrations of gastrin were elevated but no activation of histidine decarboxylase was observed. Paradoxically the enzyme-activating effect of pentagastrin was only moderately affected by glucose administration (IV).

C. Activation of the parietal cell

The injection of pentagastrin caused a marked increase in the rate of acid secretion in innervated fistula rats (VI) whereas there was no acid response to pentagastrin in chronically vagotomized fistula rats (VI). Also the acid response to histamine and insulin was abolished by vagotomy and the basal acid secretion was greatly reduced (IV, VI).

Since insulin is thought to evoke vagal excitation, the effects of insulin on gastric acid secretion were examined (IV). Insulin stimulated gastric acid secretion when injected in low doses (<1.25 U/kg). The addition of glucose to the insulin injection completely abolished the acid secretory response (IV). Higher doses of insulin (2.5 - 10 U/kg) gave no acid response. After pretreatment with 2.5 U/kg of insulin the parietal cells still responded to histamine but not to pentagastrin. After pretreatment with 5 U/kg of insulin neither histamine nor pentagastrin stimulated acid secretion.

An intact vagal innervation seems to be necessary for a normal basal secretion in the fistula rat (see above). An intact vagal innervation was important also for the acid secretion following pyloric ligation (VI). Truncal vagotomy, performed at the time of ligation, completely abolished the acid response, and unilateral vagotomy reduced it by 50 %. The acid response to pyloric ligation was not caused by distension of the stomach, since the response was unaffected by gastric drainage. The response could be blocked by atropine and chlorisondamine (a ganglionic blocking agent) but not by metiamide (an H_2-receptor blocker). When pyloric ligation was performed at various time intervals (0-8 weeks studied) after vagotomy, a gradual return of the acid response towards pre-denervation values was observed. The acid response in the chronically vagotomized rat was dependent upon distension since it was abolished by gastric drainage (VI).

In further studies on the effect of gastric distension, rats were subjected to pyloric and oesophageal

ligatures. Oesophageal ligation abolished the acid response to pyloric ligation; in innervated rats by causing oesophageal distension and in chronically vagotomized rats by preventing gastric distension (VI). Graded gastric distension caused a volume dependent acid secretory response in normal as well as in vagotomized animals, with peaks at 12 and 9 ml of distension respectively (VI). Metiamide, atropine and chlorisondamine inhibited the secretory response to distension in both normal and vagotomized animals.

7. General Discussion

Under physiological conditions gastric acid secretion is either basal (unstimulated, spontaneous, interdigestive) or stimulated (postprandial). The rate at which acid is being released under basal circumstances is thought to reflect the vagal tone, since vagotomy reduces basal acid secretion in dog (Antia et al., 1951), man (Gillespie et al., 1960) as well as in the rat (VI). Histamine is thought to participate in the physiological control of basal acid secretion since the administration of histamine H_2-receptor antagonists inhibits basal acid secretion (Black et al., 1972). Antrectomy does not seem to influence the basal acid secretion in the rat (Kowalewski, 1971), suggesting that in this species gastrin does not participate in the regulation of basal acid secretion, although a slightly reduced secretion was reported by Håkanson & Liedberg (1971a).

Physiological stimulation of gastric acid secretion by the intake of food initiates a chain of events, some of which are interdependent, some just coinciding in time.

A. Nerve-mediated stimulation of the parietal cells

The view that the vagi mediate cephalic stimulation of gastric acid secretion has long been accepted (Grossman, 1967). The vagi are thought to evoke acid secretion through direct cholinergic stimulation of the parietal cells and indirectly through release of gastrin. In several species, distension evokes gastric acid secretion (Grossman, 1962; Grötzinger, 1977) by mechanisms thought to involve vagal excition (Grossman, 1967). One way of achieving gastric distension is through pylorus ligation which also greatly stimulates acid secretion. However, the acid response to pylorus ligation in the rat does not seem to be due to distension, since it was unaffected by gastric drainage (VI). It is probably mediated by a long vago-vagal reflex (Brodie & Knapp, 1966; VI), with the receptors of the afferent part of the reflex arc located in the pyloric area (Brodie, 1966; VI). Vagotomy performed at the time of ligation abolished the acid response to pylorus ligation. A possible explanation for the abolished response could be the loss of cholinergic tone on the parietal cells, rendering them less sensitive to stimulation. With time after vagotomy the stomach regained its capacity to secrete acid in response to pylorus ligation. This effect was probably mediated by local, intramural reflexes which seem to develop gradually with time after vagotomy. The fact that atropine effectively blocked the acid response to pylorus ligation in both innervated and vagotomized rats suggests that cholinergic mechanisms are involved. In innervated rats metiamide failed to inhibit the acid response to pylorus ligation, suggesting that histamine is not involved. Ligation of the oesophagus inhibited

the acid response to pylorus ligation. In innervated rats this inhibition reflects activation of an inhibitory vagal reflex arc (cf. Brodie & Knapp, 1966), elicited by the back-up of saliva in the oesophagus; if the oesophagus was drained the acid response returned. In the denervated rats the inhibited acid response probably reflects lack of distension since saliva no longer reaches the stomach. Distension induced by instillation of 0.9 % saline into the stomach of rats subjected to ligation of both the pylorus and the oesophagus evoked copious acid secretion in both innervated and chronically vagotomized rats. The chemical messengers involved in the acid response to pylorus ligation and to gastric distension in innervated and in vagotomized rats remain to be identified.

The response to eating is initiated by conditioned reflexes (Pavlov 1898) and by visual, olfactory and gustatory impulses. The effects of cephalic stimulation can be completely abolished by vagotomy (Grossman, 1967). A widely used method for mimicking vagal activation is the induction of cerebral cytoglucopenia by drugs such as insulin or 2-deoxy-D-glucose. The insulin test has become a means for evaluating the completeness of vagotomy. Studies in man have indicated that insulin-induced hypoglycemia causes enhanced serum levels of adrenaline and gastrin (Stadil & Rehfeld, 1972; Brandsborg et al., 1975). The effects on both adrenalin and gastrin could be abolished by the combined injection of insulin and glucose (Stadil & Rehfeld, 1972), the effects on gastrin release also by β-adrenergic blockade (Christensen & Stadil, 1976). Serum concentrations of adrenalin similar to those observed

after administration of insulin cause gastrin release (Stadil & Rehfeld, 1973; Brandsborg et al., 1978). The biological importance of these observations is dubious, since adrenalin concentrations of this magnitude are not encountered in blood under physiological conditions (Brandsborg et al., 1975).

In the rat, the mechanism behing the secretory response to insulin may be somewhat different. Insulin injection caused hypoglycemia, acid secretion (only in doses of 0.2 - 1.0 U/kg) and gastrin release (1.25 - 10 U/kg). The gastric acid secretory response could be blocked by glucose administration whereas the effect on circulating gastrin levels could not (IV). Insulin stimulates acid secretion even in the absence of endogenous gastrin (Pevsner & Grossman, 1955; Olbe, 1964; Håkanson & Liedberg, 1970, 1971 b), suggesting that non-gastrinergic mechanisms contribute to the acid response after insulin injection. One such mechanism could be a direct vagal stimulation of the parietal cells since vagotomy abolishes the acid response to insulin (see for instance Lane et al., 1957). However, in the rat vagotomy abolishes also the acid response to pentagastrin and histamine (IV). There is reason to recall the statement: "The notion that stimulation of acid secretion by insulin is mediated solely by the vagus is an unproven assumption" (Grossman, 1974).

B. Gastrin-mediated stimulation of the parietal cells

Chemical stimulation of the antral mucosa has received much attention. Food (amino acids and small peptides) causes a release of gastrin (Thompson, 1969)

that is enhanced by an intact vagal innervation (Schafmayer et al., 1978). The buffering effect of food further augments the gastrin release by elevating the antral pH. Although the intragastric pH seems to influence the basal release of gastrin (Becker et al., 1973; Walsh et al., 1975; III), its main importance may be in modulating the gastrin release in response to a stimulus (Blair et al., 1978).

Endogenously released as well as injected gastrin (or pentagastrin) have several biological functions, including that of stimulating gastric acid secretion. Its acid stimulating properties vary between different species (Emås & Grossman, 1967). In the conscious fistula rat the acid-stimulating effect of gastrin is dependent upon an intact vagal innervation (V). In vagotomized rats gastrin seems to be without effect and the basal acid secretion is very low. However, a small increment in acid secretion after gastrin injection in the vagotomized rat may be masked by the buffering effect of swallowed saliva. Gastrin is capable of provoking acid secretion from the denervated fundic pouch in the rat (Lundell & Svensson, 1973), indicating a lack of dependency on vagal tone. A possible explanation could be that, in this preparation, increased firing in intramural reflex arcs sensitizes the parietal cells to gastrin.

When gastrin is released from the cell, it passes either into the circulation or into the gastric lumen (Andersson & Nilsson, 1974; Schofield et al., 1976; Fiddian-Green et al., 1978; Uvnäs-Wallensten, 1977, 1978). Among the actions of circulating gastrin are its stimulatory effects on the parietal cells and its trophic effects on the oxyntic mucosa (for reviews

see Oscarson et al., 1978; Johnson, 1979). Another physiological function of circulating gastrin is regulation of the activity and numbers of the histamine containing cells in the rat gastric mucosa (for a review, see Håkanson et al., 1974).

Luminal gastrin seems to lack the activating effect of circulating gastrin on the histamine-storing endocrine cells (V) and on the parietal cells (Hengels et al., 1980) although conflicting views have been reported (Fiddian-Green et al., 1978).

It has been argued that the parietal cells lack receptors to gastrin and that gastrin stimulates acid secretion indirectly, via intermediaries such as histamine or acetylcholine. Isolated oxyntic glands or cells responded to histamine and acetylcholine but not to pentagastrin (Michelangeli, 1974; Berglindh et al., 1976). However, evidence suggesting a direct action of gastrin have been presented by other workers. Thus, cell suspensions enriched in parietal cells were found to respond to gastrin with an increased oxygen consumption and aminopyrine uptake (Soll, 1977, 1979). The response to gastrin could not be blocked by atropine or metiamide. The addition of histamine greatly potentiated the response to gastrin. There also seems to exist a specific binding site for gastrin on gastric mucosal cell membranes (Lewin et al., 1977; Takeuchi et al., 1979). It remains to be unequivocally established that the parietal cells possess gastrin receptors and that the excitation of these receptors leads to the production of gastric acid.

C. Stimulation of the parietal cells by histamine

There is yet another link postulated between gastrin and gastric acid secretion, namely histamine. Gastrin controls the activity of the histamine-storing endocrine cells in rat gastric mucosa (Håkanson et al., 1974; I, II, IV). Histamine is a powerful stimulant of acid secretion and it has been proposed that histamine is important for the physiological regulation of acid secretion (Code 1965; Kahlson et al., 1973; Black et al., 1972). It may act alone by stimulating the parietal cells per diffusionem or via the local circulation after gastrin-induced release, or it may act in conjunction with other stimuli by sensitizing the parietal cells to the action of gastrin and to cholinergic agents (Grossman 1975). Both interpretations explain why histamine H_2-receptor antagonists are effective in inhibiting basal acid secretion as well as secretion stimulated by gastrin (Grossman & Konturek, 1974; Thjodleifsson & Wormsley, 1975). Neither explains why vagotomy abolishes the acid response to gastrin, insulin and histamine, unless also acetylcholine is released (by the vagus) in the vicinity of the parietal cells, to sensitize them in a fashion similar to that postulated for histamine. On the whole, if histamine is at all involved, the theory of permissive action (Grossman, 1975) seems more consistent with available data.

There is no convincing evidence that histamine mediates the acid response to vagal nerve stimulation (see e.g. II and VI). There is considerable doubt that gastrin-induced acid secretion is mediated by histamine (see Johnson & Aures 1970; El Munshid et

al., 1976, 1980a,b). Histamine may perhaps play a permissive role, enabling the parietal cells to respond to gastrin (see Håkanson et al., 1979).

This study was supported by the Swedish Medical Research Council (04x-1007, 14x-4144), the Medical faculty of the University of Lund and the Albert Påhlsson Foundation.

The expert technical assistance of Anna Themner is gratefully acknowledged.

8. Summary

1/ The following intraluminal factors affect the gastrin cells:

- the chronically elevated antral pH causes an increased rate of gastrin release

- the combined effects of food passage and high antral pH cause a proliferation of gastrin cells

The following intraluminal factors do not affect the activation of the histamine-containing cells:

- pH changes do not affect the activation induced by gastrin

- luminal gastrin does not activate histidine decarboxylase

2/ The acid response to pyloric ligation is mediated by a long vago-vagal reflex independently of gastric distention. After vagotomy intramural reflexes have the potential to develop and mediate an acid response to distension.

3/ Low doses of insulin cause a vagally mediated acid secretory response. Higher doses of insulin do not stimulate gastric acid secretion although the serum gastrin concentration and the gastric histidine decarboxylase activity are high. When hypoglycemia is prevented, insulin-induced gastrin release is not accompanied by an activation of the histamine-storing endoscrine cells. Exogenous gastrin retains the capacity to activate the enzyme.

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EFFECTS OF INSULIN ON THE SERUM GASTRIN CONCENTRATION, GASTRIC ACID SECRETION AND HISTAMINE MOBILIZATION IN THE RAT

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1980

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SUMMARY

1. Small doses of insulin (< 1.25 U/kg) caused hypoglycemia, stimulated gastric acid secretion slightly and activated gastric mucosal histidine decarboxylase moderately. The pentagastrin-induced activation of histidine decarboxylase was enhanced by insulin in these doses.

2. Large doses of insulin (> 2.5 U/kg) caused marked hypoglycemia, hypergastrinemia and high incidence of gastric ulcers. In addition, it lowered gastric mucosal histamine concentration and greatly activated histidine decarboxylase. Gastric acid secretion was not stimulated. Pentagastrin-stimulated acid secretion was blocked and histamine-stimulated secretion inhibited to a lesser degree.

3. Combined administration of insulin and glucose prevented the acid response (insulin dose 0.6 U/kg) and the activation of histidine decarboxylase but not the hypergastrinemia (2.5 U/kg). Paradoxically, combined treatment with insulin and glucose did not prevent pentagastrin from activating histidine decarboxylase. Glucose alone raised the serum gastrin concentration in both unoperated and vagotomized rats. The histidine decarboxylase activity was not raised. Glucose greatly inhibited the pentagastrin-induced release of histamine but inhibited the pentagastrin-induced enzyme activation only moderately.

4. In vagally denervated rats, insulin (5 U/kg) caused a slight increase in the serum gastrin concentration but the histidine decarboxylase activity was not affected.

5. We interpret the results as follows: With low doses of insulin the gastric secretagogue effect is attributable mainly to vagal nerve stimulation elicited by the hypoglycemia. With high doses of insulin there is no acid response. Apparently the effects of the ensuing hypergastrinemia are counteracted by a mechanism that prevents gastrin from stimulating the parietal cells. This unknown mechanism does not prevent gastrin from releasing gastric histamine and stimulating gastric histidine decarboxylase. Hyper-

glycemia, by an unidentified mechanism, prevents gastrin from activating histidine decarboxylase. The insulin-induced release of gastrin is not elicited exclusively through vagal excitation since insulin releases gastrin also in combination with hyperglycemia or euglycemia. The great enzyme-activating effect of insulin is only partly a consequence of insulin-induced gastrin release. In addition, insulin enhances the responsiveness to gastrin. This seems to be a consequence of vagus nerve stimulation, since there is no insulin-induced enhancement of the response to gastrin in vagotomized rats.

INTRODUCTION

Insulin hypoglycemia is thought to cause vagal excitation. Hence, insulin is often used to simulate the cephalic phase of gastric secretion. However, since vagal excitation causes release of gastrin (Olbe, 1966) and probably also of other biologically active peptides, all effects elicited by vagal excitation on the parietal cells may be either direct or indirect, produced by agents released by the vagal excitation. Insulin hypoglycemia is known to raise the concentration of circulating gastrin. It is also known to raise the histidine decarboxylase activity and to reduce the histamine content of the rat stomach (Kim & Shore, 1963; Kahlson, Rosengren, Svahn & Thunberg, 1964; Kim, Ridley & Tuegel, 1968). Whether these effects are exerted by the vagus nerve stimulation directly as claimed by Lundell (1976), or indirectly by the gastrin released (Håkanson & Liedberg, 1971) is debated. The effects of exogenous insulin on gastrin release, gastric acid output and histamine mobilization were studied in the rat. The following questions prompted the study: 1) Are the effects of insulin direct or elicited by the hypoglycemia? 2) Are the effects of hypoglycemia direct or elicited by vagal excitation? 3) Are the effects of vagal excitation direct or elicited by gastrin?

MATERIALS AND METHODS

Animals. Male rats of the Wistar or Sprague-Dawley strains were used. Total abdominal vagotomy was achieved by cutting both vagal trunks immediately below the diaphragm and dissecting the oesophageal wall for small nerve fibers. A pyloroplasty was made at the same time to prevent gastric dilatation. The rats used for determination of histidine decarboxylase activity weighed 150-250 g, those used for studies on gastric acid secretion 250-350 g. They were fed a standard diet of rat food pellets (SAN-bolagen, Sweden) and tap water. Before sacrifice the rats were kept in individual cages with wire mesh bottoms and deprived of food but not water for 40-48 hours. After sacrifice the stomachs were inspected for gastric ulcers. Plastic gastric fistulas, modified after Lane, Ivy & Ivy (1957) and Bel, Leurat, Nesmos & Girard (1966) were implanted into the rumen, permitting repeated

studies of gastric secretion in the same group of rats during different experimental conditions for periods of time up to three or four months. These animals were fasted for 24 hours before the experiments.

Drugs. Insulin (Vitrum, Sweden) was given subcutaneously in doses from 0.1-10.0 U/kg. Also, pentagastrin (Peptavlon, ICI, England) and histamine dihydrochloride (Fluka, Basle, Switzerland) were given subcutaneously, 500 µg/kg and 25 mg (free base)/kg, respectively. Glucose was given intraperitoneally and/or subcutaneously (as specified).

Determination of serum gastrin concentrations. The rats were anesthetized with diethyl ether, the abdomen was opened and blood was drawn from the aorta. Serum was lyophilized and stored at -20°C until analyzed for gastrin using a radioimmunoassay for human gastrin (Stadil & Rehfeld, 1971, 1973). The details of this procedure as applied to rat serum have been given in a previous communication (Håkanson, Kroesen, Liedberg, Oscarson, Rehfeld & Stadil, 1974).

Determination of serum glucose concentrations. Glucose was determined enzymatically by the glucose oxidase method (Marks, 1959).

Determination of gastric histidine decarboxylase activity. The stomachs were cut open along the greater curvature and the mucosal surface was cleaned with 0.9% saline. The mucosa of the oxyntic gland area was homogenized in 0.1 M phosphate buffer, pH 6.9-7.0, to a final concentration of 100 mg (wet weight) per ml. Aliquots of 0.5 ml of the homogenates were incubated with 1-^{14}C-L-histidine in the presence of pyridoxal-5-phosphate at 37°C for 1 hr under nitrogen. Total reaction volume was 0.53 ml. The amount of $^{14}CO_2$ formed during the reaction was measured by liquid scintillation counting (Håkanson et al., 1974; Håkanson, Larsson, Liedberg, Rehfeld & Sundler 1977b).

Determination of gastric histamine concentration. The gastric mucosal homogenates were mixed with equal volumes of 3 per cent trichloroacetic acid and stored in a refrigerator over-night after which precipitated material was spun down by centrifugation at low speed. The supernatants

were diluted 1:50 with redistilled water. The histamine content of these deproteinized extracts was measured fluorometrically without further purification (cf. Håkanson & Rönnberg, 1974).

Determination of gastric acid output. The fistula rats were thoroughly familiarized with the experimental situation before being used for actual collection of gastric juice. Under light diethyl ether anestesia the stomachs of the fasted fistula rats were rinsed with warm 0.9% saline until the return was clean, and 10 ml 0.9% saline was injected subcutaneously to compensate for subsequent loss of fluid. The rats were then transferred to Bollman-type restraining cages where they quickly regained consciousness. The fistula was then allowed to drain freely for one hour after which gastric juice was collected in one-hour portions for up to 6 hours. The volume of each one-hour portion was measured and the acid output determined by titration with 0.02 N NaOH; phenolphthalein was used as indicator.

RESULTS

I. Effects of graded doses of insulin

1. Gastric acid secretion. Only low doses of insulin, 0.2-1.25 U/kg, produced a significant acid response which appeared 2-3 hours after the injection (Fig. 1). Maximum response was obtained with 0.6 U/kg (Fig. 2). Higher insulin doses either had no effect or suppressed basal acid secretion. With doses of 5 and 10 U/kg the mortality was 17 and 26 per cent respectively (calculated from experiments on 40 fistula rats). In addition, approximately 50 per cent of the surviving rats had convulsions or were prostrate or comatose at the highest dose level. Pretreatment with insulin, 2.5 U/kg, prevented pentagastrin-stimulated but not histamine--stimulated acid secretion (Fig. 3). A higher dose, 5 U/kg, blocked both pentagastrin- and histamine-stimulated acid secretion (not shown in figure).

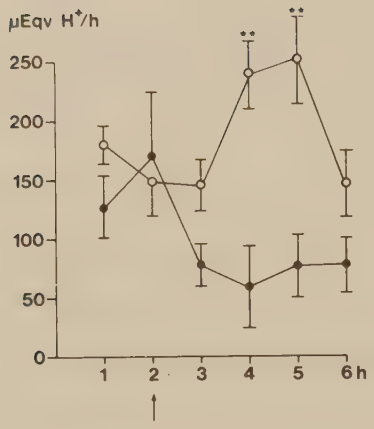

*Fig.1. Acid secretory response to a subcutaneous injection of insulin, 0.6 U/kg, with(●) or without (o) the simultaneous ip injection of 1.5 ml 50% glucose. The experiments were performed with one week's interval on the same group of 8 fistula rats weighing 250-300 g. Injections were made at arrow. Vertical bars give S.E. of the mean. Stimulated acid output is compared to the basal acid secretion (second hour of collection) using Student's t-test for paired observations. ** for $0.001 < p < 0.01$.*

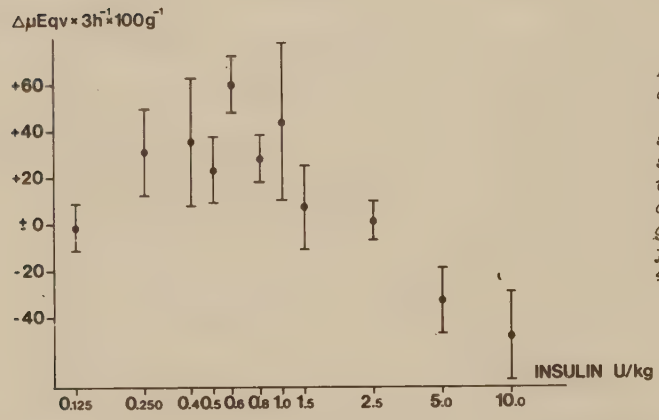

Fig.2. Gastric acid output as change relative to baseline level, following sc administration of stepwise increasing amounts of insulin. Each value is the mean of 11-35 determinations. Vertical bars give S.E. of the mean. Forty fistula rats, weighing 200-400 g, were used in this study.

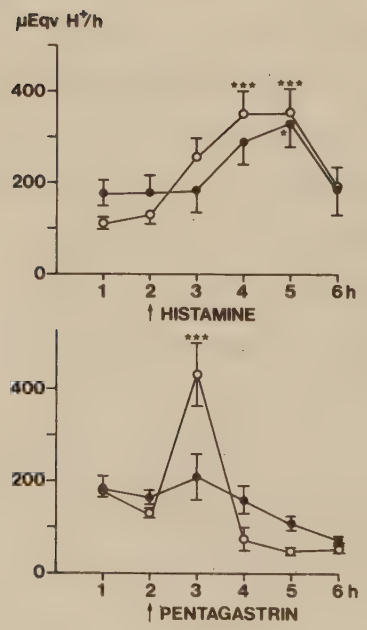

*Fig.3. Acid secretory response to subcutaneous injection of pentagastrin, 500 µg/kg (top) or histamine dihydrochloride, 25 mg/kg (below) before (o) and after (●) pretreatment with insulin, 2.5 U/kg sc. The experiments were performed with one week's interval on the same group of 10 rats. Five received pentagastrin and 5 histamine. Insulin injection was given at zero time. Injections of histamine or pentagastrin were made at arrow. Vertical bars give S.E. of the mean. Stimulated acid output is compared to the basal acid secretion (second hour of collection) using Student's t-test for paired observations. *** for $p < 0.001$, * for $p < 0.05$.*

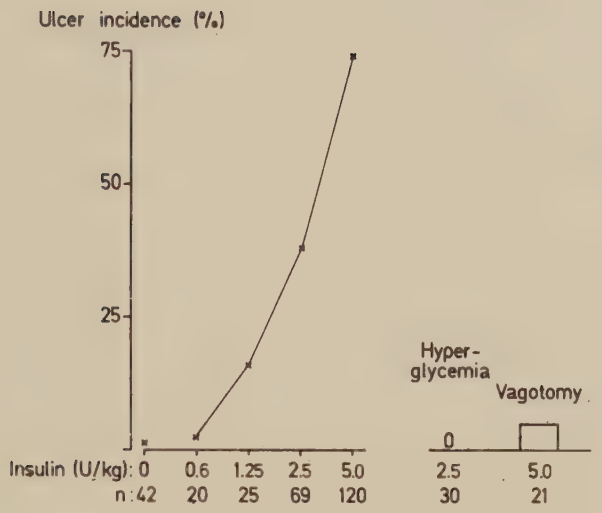

Fig.4. The incidence of gastric ulcer(expressed as percentage of the rats afflicted) as related to the insulin dose in un-operated rats (left). In glucose-loaded rats and in vagally denervated rats insulin (5 U/kg) is much less effective in producing ulcers. The glucose administration protocol was as in Table 6. The stomachs were inspected for ulcers 3-5 hours after the sc injection of insulin.

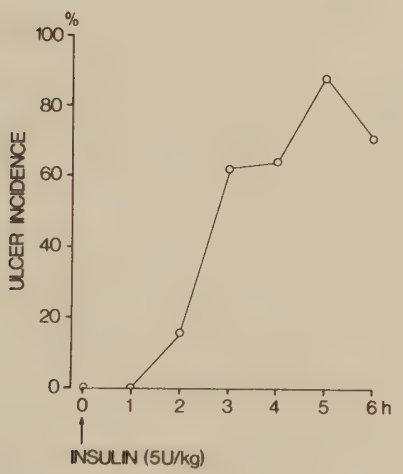

Fig.5. Time course of ulcer generation following a subcutaneous injection of insulin (5 U/kg).

2. Ulcer incidence. Ulcerations were frequently found in the oxyntic mucosa of insulin-treated rats. They were single or multiple, and they were never seen outside the oxyntic gland area. The incidence of gastric ulcer increased with increasing doses of insulin (Fig. 4). With 5 U/kg of insulin the ulcer incidence was 75 per cent. The ulcers developed soon after the injection of insulin. Maximal ulcer incidence was observed after 5 hours (Fig. 5).

3. Serum glucose concentrations. There was a marked decrease in serum glucose concentration 1 hour following administration of insulin (Table 1). The decrease was dose-dependent. With doses below 5 U/kg the decrease was followed by hyperglycemia after 4-5 hours. This rebound hyperglycemia was manifest only with the lower doses, conceivably because with the higher doses the hyperglycemic reaction occurred after more than 6 hours, the latest time studied.

4. Serum gastrin concentrations. The serum concentration of gastrin started to increase 1-2 hours after the administration of insulin in doses of 0.6 U/kg and higher (Table 2). The peak values were reached after 2-4 hours. A dose of 5 U/kg gave maximal response. Low doses of insulin, 0.3 U/kg, did not significantly raise the serum gastrin level.

5. Gastric histidine decarboxylase activity. All doses of insulin (5 U/kg was a near-maximal dose) raised the gastric histidine decarboxylase activity (Table 3); the increase was notably slow in onset and peak values were not reached until 4-5 hours after the injection. Following pretreatment with insulin the pentagastrin-induced enzyme activation was enhanced (Fig. 6).

6. Gastric histamine concentration. Insulin doses of 2.5 U/kg and higher lowered the mucosal histamine concentration by approx. 30 per cent (Table 4). The effect was maximal 3-5 hours after the injection. Doses lower than 1.25 U/kg had no effect on the gastric histamine content.

Table 1

Effect of insulin on serum glucose concentration (mmol/l)

	Controls 5.4 ± 0.4 (30)					
	Insulin dose (U/kg)					
Time after injection (h)	0.15	0.3	0.6	1.25	2.5	5.0	10.0
1	2.2±0.5 (3)	1.2±0.3 (6)**	2.6±0.2 (12)***	2.2±0.1 (10)***	2.3±0.5 (7)***	2.2±0.3 (18)***	0.5±0.1 (5)***
2	1.8±0.4 (3)	1.3±0.2 (6)***	4.3±0.2 (7)	3.8±0.3 (10)**	2.8±0.3 (7)***	2.1±0.2 (23)***	0.3±0.1 (5)***
3	2.5±0.3 (3)	2.3±0.5 (7)	4.8±0.5 (22)	6.9±0.6 (5)	4.6±0.9 (12)	1.6±0.2 (26)***	0.6±0.1 (5)***
4	4.1±0.3 (9)	4.7±0.4 (17)	4.9±0.4 (17)	5.7±0.6 (15)	6.2±0.6 (7)	2.8±0.4 (22)***	1.6±0.2 (5)***
5	4.4±0.3 (3)	4.4±0.6 (11)	6.0±0.7 (11)	9.6±0.4 (5)	8.6±1.0 (7)	4.0±0.8 (18)	2.1±0.6 (5)***
6	4.0±0.4 (3)	3.6±0.3 (3)	6.4±0.8 (5)	9.1±0.4 (5)	9.3±1.0 (7)	4.9±0.6 (14)	3.8±0.3 (5)**

Mean ± S.E. of mean (n)

Statistical symbols indicate reduction compared with controls. ** for $p < 0.01$; *** for $p < 0.001$.

Table 2

Effect of insulin on serum gastrin concentration (pg eqv. SHG/ml)

	Controls 48 ± 2.3 (79)				
	Insulin dose (U/kg)				
Time after injection (h)	0.6	1.25	2.5	5.0	10.0
1	60±9 (15)	63±7 (16)*	55±8 (18)	75±6 (29)***	77±12 (18)**
2	50±8 (13)	62±6 (13)*	59±10 (18)	112±10 (31)***	81±13 (18)**
3	45±6 (14)	61±7 (7)	72±11 (28)*	122±14 (36)***	89±14 (21)**
4	45±4 (9)	57±7 (13)	72±11 (19)*	109±15 (28)***	76±6 (21)***
5	51±3 (14)	53±7 (13)	70±18 (31)	76±8 (35)***	75±12 (21)*
6	44±6 (11)	48±5 (13)	68±5 (21)***	72±14 (24)*	71±17 (14)

Mean ± S.E. of mean (n)

Statistical symbols indicate increase over controls. * for $p < 0.05$; ** for $p < 0.001$; *** for $p < 0.001$.

Table 3

Effect of insulin on gastric histidine decarboxylase activity (pmol CO_2/mg/h)

	Controls	5.8 ± 0.3 (55)				
	Insulin dose (U/kg)					
Time after injection (h) 0.15	0.3	0.6	1.25	2.5	5.0	10.0
1 1.7±0.4 (3)	6.2±1.2 (14)	6.9±1.9 (16)	5.8±0.7 (10)	9.5**±1.4 (18)	9.2**±1.1 (29)	12.1***±1.8 (14)
2 2.5±0.6 (3)	10.4**±1.6 (9)	11.3*±2.4 (17)	14.8***±2.5 (10)	13.5***±1.2 (18)	18.3***±1.7 (42)	16.2***±1.7 (21)
3 5.0±1.0 (3)	12.6***±1.3 (13)	13.9***±1.3 (23)	18.7***±2.4 (10)	26.3***±3.4 (21)	27.2***±2.2 (33)	32.4***±2.9 (14)
4 4.8±1.4 (3)	12.9***±1.7 (13)	14.0***±1.6 (23)	30.2***±5.9 (10)	31.3***±2.2 (23)	34.6***±3.3 (18)	50.1***±7.0 (9)
5 2.9±0.6 (3)	13.4***±2.1 (16)	15.0***±2.1 (20)	28.9***±2.0 (10)	40.1***±3.5 (21)	41.9***±2.6 (29)	50.0***±5.2 (18)
6 2.7±1.0 (4)	9.9**±1.2 (7)	14.6***±1.7 (6)	18.8***±2.6 (5)	28.2***±2.5 (18)	42.6***±3.7 (24)	50.6***±6.3 (11)

Mean ± S.E. of mean (n)

Statistical symbols indicate increase over controls. * for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$.

Table 4

Effect of insulin on gastric mucosal histamine concentration (µg/g)

	Controls	59 ± 2.1 (54)				
	Insulin dose (U/kg)					
Time after injection (h) 0.15	0.3	0.6	1.25	2.5	5.0	10.0
1 61±0.3 (3)	66±7.7 (12)	53±4.2 (22)	48***±2.4 (22)	44***±3.3 (13)	50*±3.0 (34)	50*±4.0 (12)
2 58±3.7 (3)	64±6.2 (16)	66±4.8 (17)	49**±2.9 (18)	45***±2.3 (25)	48**±3.4 (33)	50**±1.9 (23)
3 47±0.9 (3)	51*±3.1 (22)	56±2.8 (17)	57±4.9 (13)	43***±2.6 (18)	43***±2.6 (33)	41***±2.6 (21)
4 64±9.0 (3)	51±4.4 (19)	66±3.9 (17)	50**±2.6 (23)	50**±2.6 (23)	46**±3.2 (34)	41***±2.5 (21)
5 80±3.0 (3)	54±2.2 (20)	72±5.4 (17)	51±4.6 (10)	55±6.1 (23)	40***±2.4 (49)	41***±1.7 (13)
6 64±6.7 (3)	63±6.8 (16)	72±5.6 (17)	51*±2.3 (10)	56±2.3 (18)	44**±4.1 (27)	43***±3.2 (18)

Mean ± S.E.M. (n)

Statistical symbols indicate reduction compared with controls. * for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$.

Table 5

Effect of glucose on serum gastrin concentration and gastric histidine decarboxylase activity in vagotomized and unoperated rats

	Unoperated rats			Vagotomized rats		
Treatment	Serum glucose concentration, mM	Serum gastrin concentration, pg SHG eqv/ml	Gastric histidine decarboxylase activity, pmol CO_2/mg/h	Serum glucose concentration, mM	Serum gastrin concentration, pg SHG eqv/ml	Gastric histidine decarboxylase activity, pmol CO_2/mg/h
Saline	6.2±0.3 (6)	51±7 (15)	4.4±1.0 (12)	4.2±0.7 (7)	141±25 (7)	79.4±12.9 (7)
Glucose	37.7±3.1*** (11)	167±20*** (32)	6.5±1.1 (24)	74.4±1.2*** (10)	303±27*** (10)	62.5±13.0 (15)

Mean ± S.E.M. (n).

Statistical symbols indicate difference between saline- and glucose-treated rats. *** for $p < 0.001$.

The rats were of the Sprague-Dawley strain and weighed 250-300 g. Glucose administration protocol: 1 ml ip and 1 ml sc of 50% glucose was given hourly three times. The rats were killed one hour after the last injection.

Table 6

Effects of insulin, alone or in combination with glucose, on serum glucose, serum gastrin and gastric histidine decarboxylase.

Treatment	Serum glucose conc., mM		Serum gastrin conc., pg SHG eqv./ml		Gastric histidine decarboxylase activity, pmol CO_2/mg/h	
Saline	7.4 ± 0.4 (8)	N.S.	43 ± 7 (11)	*	4.5 ± 0.5 (12)	***
Insulin, 2.5 U/kg, † 5 h	8.1 ± 1.1 (4)		132 ± 40 (4)		63.8 ± 13.9 (4)	
Insulin + glucose, 1x, † 1 h	13.2 ± 2.3 (8)		81 ± 11 (10)		3.0 ± 0.7 (10)	
, 2x, † 2 h	49.3 ± 4.9 (7)	***	122 ± 25 (9)	N.S.	6.2 ± 0.4 (10)	**
, 3x, † 5 h	58.8 ± 0.6 (8)		174 ± 46 (12)		9.1 ± 1.5 (13)	

Mean ± S.E. of mean (n).

Statistical symbols indicate differences between groups as indicated. * for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$.

The rats were of the Sprague-Dawley strain and weighed 250-300 g.

Glucose administration protocol: 1.5 ml 50% glucose was given intraperitoneally simultaneously with insulin s.c. One hour later some of the rats were killed. The others received 0.5 ml intraperitoneally and 1 ml subcutaneously. Another hour later another group of rats was sacrificed. The surviving rats received 0.5 ml intraperitoneally and 0.5 ml subcutaneously. These rats were killed 5 hours after the first injection.

II. Effect of glucose alone and of insulin + glucose

In unoperated and in vagotomized rats glucose raised the serum gastrin concentration but not the histidine decarboxylase activity (Table 5). Glucose administration greatly suppressed the pentagastrin-induced release of histamine; the activation of histidine decarboxylase was only moderately inhibited (Fig. 7).

Combined administration of insulin (0.6 U/kg) and glucose in doses that produced hyperglycemia prevented the acid response (Fig. 1) and prevented insulin from inducing gastric ulcers (Fig. 4). Glucose did not prevent the insulin-induced increase in serum gastrin (Table 6). Paradoxically, the activation of histidine decarboxylase was almost completely abolished although the response to pentagastrin was enhanced (Fig. 8).

Combined administration of insulin (2.5 U/kg) and glucose in doses that prevented the insulin-induced hypoglycemia (euglycemic response) raised the serum gastrin concentration over controls without activating histidine decarboxylase (Fig. 9).

III. Effect of insulin in vagotomized rats

Following vagal denervation basal gastric acid secretion was much reduced (Fig. 10), the serum gastrin concentration was increased as was the gastric histidine decarboxylase activity (Table V; see also Håkanson and Liedberg, 1971). Insulin did not stimulate acid secretion, nor did pentagastrin and histamine (Fig. 10). Insulin (5 U/kg) raised the serum gastrin level slightly ($p < 0.05$) without activating histidine decarboxylase (Fig. 11). In vagotomized rats the ulcer incidence was greatly reduced compared to unoperated rats (Fig. 4).

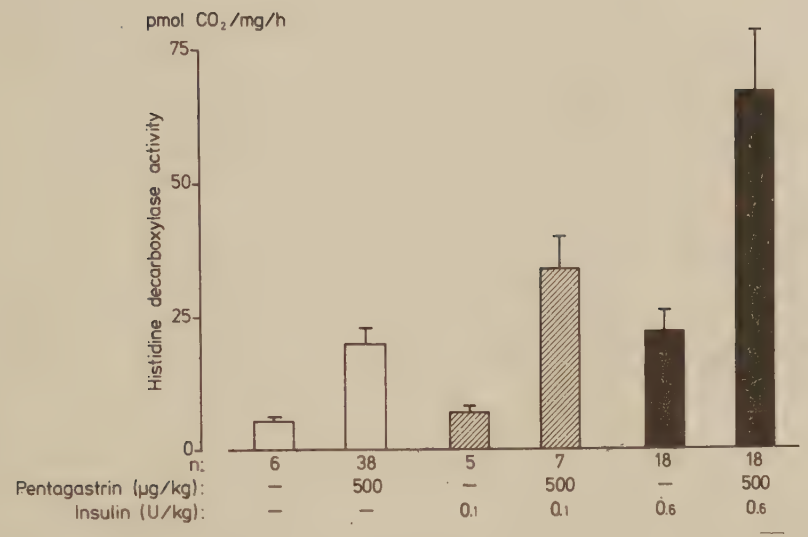

Fig.6. Gastric histidine decarboxylase activity following administration of pentagastrin or insulin or combinations thereof. All injections were given subcutaneously. The rats (fasted for 48 hours) were killed one hour after pentagastrin and four hours after insulin administration.

DISCUSSION

The present results show that insulin is a poor secretagogue in the conscious fistula rat and that only doses below 1.5 U/kg stimulate gastric acid secretion (see also Lane et al. 1957; Kim et al. 1968). Since under these circumstances the rise in serum gastrin concentration was moderate, acid secretion was probably in part stimulated by the vagus directly. Vagal excitation is thought to be a consequence of the hypoglycemia that follows upon insulin injection (Gregory, 1962). Injection of glucose in amounts that prevented hypoglycemia also prevented the acid response, an observation which seems to favour the idea of vagal involvement in the insulin-induced excitation of the parietal cells. However, insulin in doses above 1.25 U/kg gave no secretory response despite the hypoglycemia. Very high insulin doses (5 U/kg and higher) actually suppressed gastric acid secretion (see also Hirschowitz & O'Leary, 1964), although the concentration of circulating gastrin was high. It could be shown that high doses of insulin prevented the acid response to pentagastrin while being

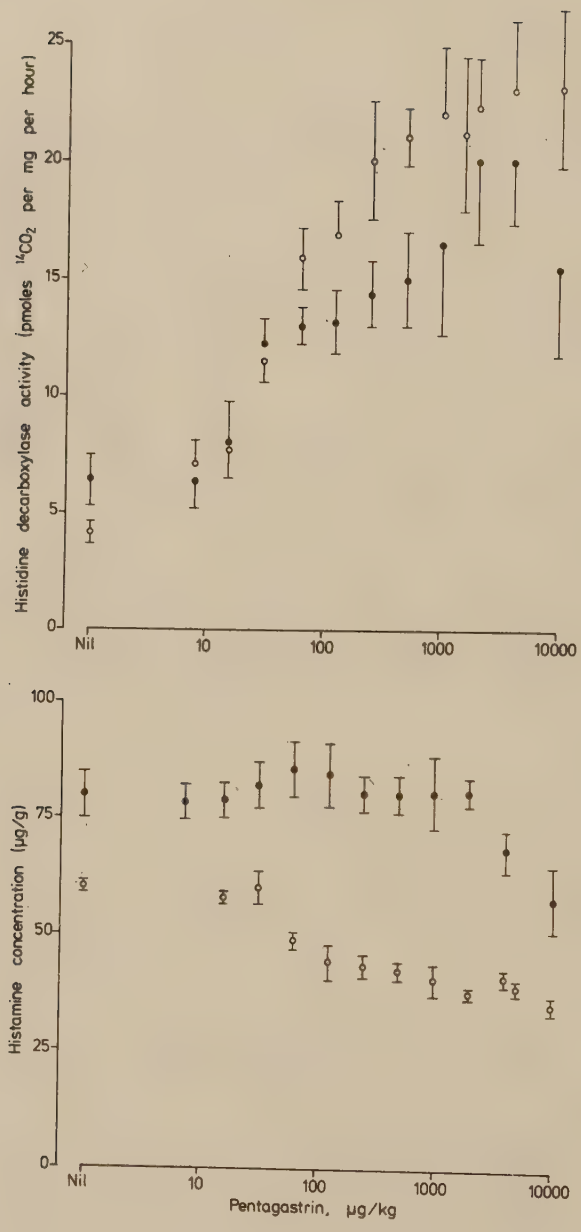

Fig.7. Histidine decarboxylase activity and mucosal histamine concentration in response to increasing doses of pentagastrin in untreated rats (o) and in rats pretreated with glucose(●). Glucose was given as a 50% solution: 1 ml ip and 1 ml sc followed by three sc injections of 1 ml with hourly inervals. The animals were killed one hour after the last injection. Pentagastrin was given sc one hour before sacrifice. Nil insicates basal enzyme activity. Mean and S.E. of the (vertical bars) are calculated from six to forty determinations.

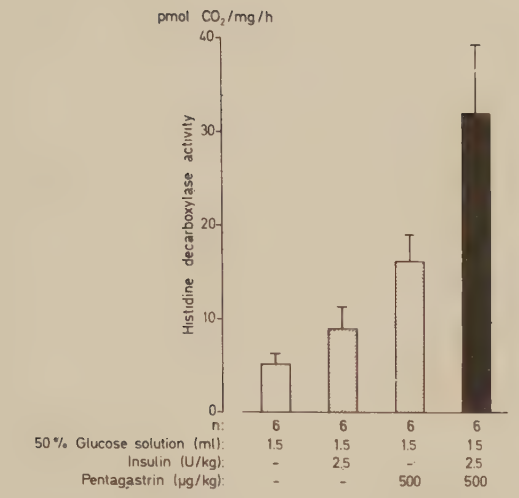

Fig.8. Gastric histidine decarboxylase following adminini-stration of glucose, insulin or pentagastrin or combinations thereof. The rats (fasted for 48 hours) were of the Sprague-Dawley strain and weighed 200-250 g. Glucose was given subcutaneously every hour for four hours. The rats were killed five hours after the first injection. Insulin was injected subcutaneously at the same time as the first glucose injection. Pentagastrin was injected subcutaneously one hour before sacrifice.

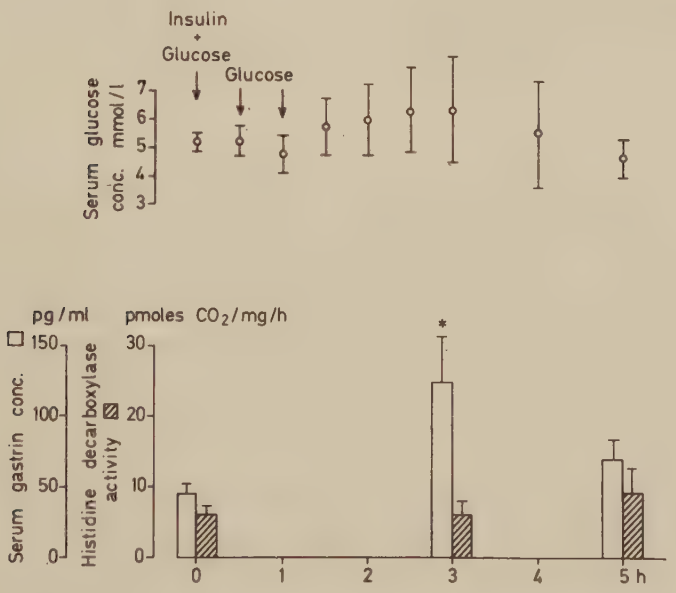

Fig.9. Effect of euglycemia following combined treatment with insulin (2.5 U/kg) and glucose on the serum gastrin concentration and the histidine decarboxylase activity in twenty rats weighing 200 g. Glucose administration protocol: 1.1 ml 50% glucose subcutaneously together with insulin at zero time, 0.4 ml glucose at 30 min and at 60 min after the first inj.

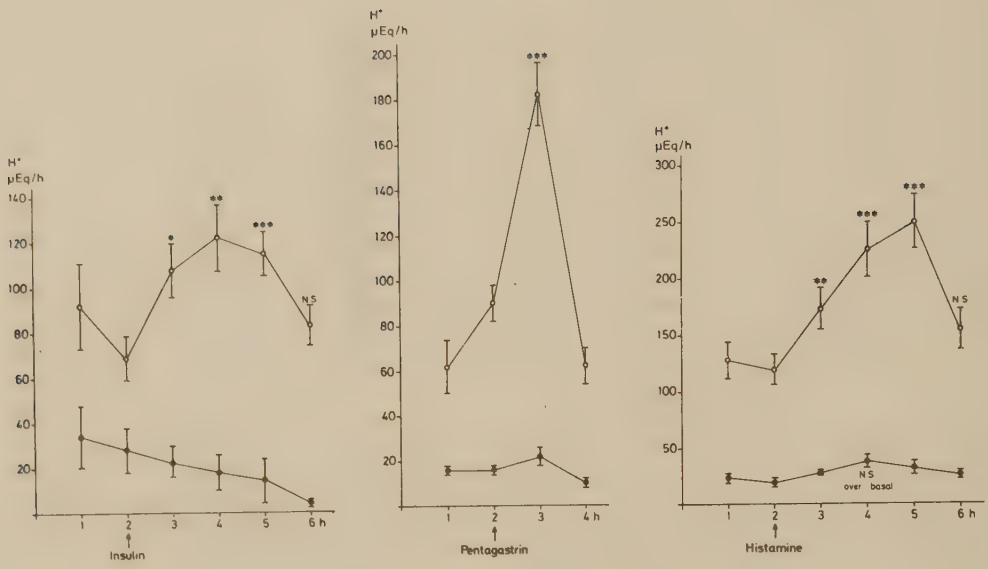

Fig.10. Acid secretory response to the sc injection of insulin (0.6 U/kg), histamine dihydrochloride (25 mg/kg) and pentagastrin (125 µg/kg) in normal (o) and in vagally denervated (•) fistula rats. Each value represents the mean of from 6 to 38 determinations. Vertical bars give S.E. of the mean. Stimulated acid output is compared to the basal acid secretion (second hour of collection).
**** for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$.*

less inhibitory to the acid response to histamine (see also Hirschowitz & Robbins, 1966). Marked hypoglycemia may depress gastric secretion and consequently mask the secretagogue action of insulin, but Hirschowitz & Robbins (1966) seem to be of the opinion that insulin acts directly on the gastric mucosa. Administration of insulin may elicit complex changes in gastric functions that cannot be explained simply by effects of vagal excitation.

Before attempting to answer the three questions that prompted our study we were thus presented with a fourth question: What blocks the acid-stimulatory effect of gastrin released by high doses of insulin? No definite answer can be given at present. The hypothetical agent is more effective in inhibiting pentagastrin-stimulated than histamine-stimulated acid secretion.

The time-course of the insulin-induced activation of gastric histidine decarboxylase seems to parallel the rise in serum gastrin concentrations. It is notable, however, that also relatively low insulin doses, incapable of raising the serum gastrin concentration, activated the histidine decarboxylase. Hence, a direct vagal pathway may activate the enzyme. However, this observation may also be explained by insulin enhancing the responsiveness to gastrin. Earlier results indicate that the vagal contribution to the enzyme activation is insignificant (Håkanson, Hedenbro, Liedberg, El Munshid & Rehfeld, 1977a).

How does insulin release gastrin? Activation by insulin of gastric histidine decarboxylase seems to be dependent upon the hypoglycemia, suggesting at first that hypoglycemia is essential for the insulin-induced release of gastrin. However, pretreatment with glucose did not prevent insulin from releasing gastrin - on the contrary, glucose per se released gastrin - but it did prevent the enzyme activation. This apparent paradox can be resolved by assuming that glucose somehow counteracts the enzyme-activating effect of gastrin. This agrees with the finding that in vagotomized rats glucose raised the serum gastrin concentration without raising the histidine decarboxylase activity. It disagrees, however, with the finding that glucose inhibited pentagastrin-induced enzyme activation only moderately. At present, there is no obvious explanation for this discrepancy. Also the mechanism whereby insulin releases gastrin must remain unexplained. It seems unlikely that the hypoglycemia (or the vagal excitation for that matter) is responsible since gastrin was released just as effectively by insulin + glucose.

The questions that prompted this study can be tentatively answered as follows: 1) Are the effects of insulin direct or elicited by the hypoglycemia? With low doses of insulin the gastric secretagogue effect can be abolished by hyperglycemia and since the serum gastrin concentration was only moderately increased by these doses of insulin this secretagogue effect may therefore well be direct. With high doses of insulin the serum

gastrin concentration was raised much higher and the histidine decarboxylase was greatly activated. The enzyme activation was prevented by glucose in doses producing hyperglycemia or euglycemia but the release of gastrin was not. Apparently, the increased serum gastrin concentration does not lead to enzyme activation because glucose impairs the gastrin-induced enzyme activation. Thus, gastrin release is a direct action of insulin, not mediated by the hypoglycemia.

2) Are the effects of hypoglycemia direct or elicited by vagal excitation? It is probable (but not certain) that vagal excitation caused the increase in acid output observed with low doses of insulin. It is difficult to prove in rats because vagal denervation renders the parietal cells virtually insensitive to all stimuli, including gastrin (see also Håkanson & Liedberg, 1971). The part played by the vagus was examined in vagally denervated rats. In such animals insulin in high doses raised the serum gastrin concentration; the histidine decarboxylase activity was not raised. These results suggest that vagal excitation is not involved in the gastrin release induced by high doses of insulin.

3) Are the effects of vagal excitation direct or elicited by gastrin? We have previously argued that gastrin is the sole physiological stimulant of gastric histidine decarboxylase (Håkanson & Liedberg, 1971; Håkanson et al. 1977a). On the whole, the results of this study support this view. However, low doses of insulin (0.6 U/kg) activate histidine decarboxylase despite the fact that the serum gastrin concentration is not elevated. This finding does not necessarily mean that vagal excitation per se is responsible. The enzyme activation following low doses of insulin may be explained by an enhanced sensitivity to gastrin as a result of insulin pretreatment. Thus, the normal vagal impulse flow seems to be of importance for the maintenance of a proper responsiveness to gastrin, excitation enhancing and denervation reducing this responsiveness. We conclude that

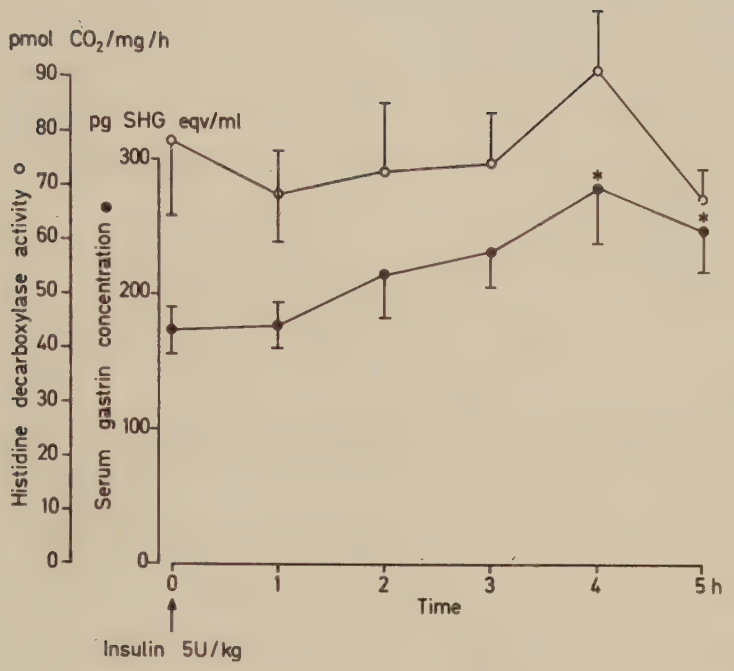

Fig.11. Effect of insulin on the serum gastrin concentration and the gastric histidine decarboxylase activity in vagally denervated rats (fasted for 48 hours). Each value is the mean of 19-27 determinations. Vertical bars give S.E. of the mean.

activation by insulin of gastric histidine decarboxylase is caused by circulating gastrin but that the magnitude of this response is determined by the sensitivity to gastrin. The sensitivity to gastrin is perhaps partly determined by the vagal impulse flow.

ACKNOWLEDGEMENTS

Grant support from the Swedish Medical Research Council (04X-1007).

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LUMINAL FACTORS IN THE ACTIVATION OF HISTIDINE DECARBOXYLASE IN RAT GASTRIC MUCOSA.

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1980

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Abstract:

Fasted rats have a low gastric histidine decarboxylase activity. Intravenous infusion of heptadecapeptide gastrin for two hours raised the enzyme activity. Intragastric perfusion with the same dose of gastrin-17 and for the same period of time did not reproduce the effect of circulating gastrin-17. The enzyme activating effect of intravenous gastrin-17 was not affected by the intragastric pH (from 2.5 to 7.0).

Introduction:

Immunoreactive gastrin is released into the gastric lumen in several species (Jordan & Yip, 1972; Andersson & Nilsson, 1974; Schofield et al., 1976; Fiddian-Green et al., 1978; Uvnäs-Wallensten, 1978). There have been speculations that luminal gastrin may be of physiological importance and exert some of the effects of circulating gastrin, i.e. stimulation of the parietal cells (Morrell & Keynes, 1975; Fiddian-Green et al., 1978) and trophic effects on the digestive tract (Johnson et al., 1978). Another function of circulating gastrin is to activate the histamine-storing endocrine cells in rat gastric mucosa (Håkanson et al., 1974). This activation is reflected in an increased activity of the histamine-forming enzyme histidine decarboxylase. Elevation of the intragastric pH leads to gastrin release (Alumets et al., 1980) and to activation of histidine decarboxylase (Håkanson & Liedberg, 1970). The present study was prompted by the following two questions: 1/ Is luminal gastrin effective in activating histidine decarboxylase?, and 2/ Does the intragastric pH influence the sensitivity to gastrin of the histamine-storing endocrine cells?

Materials and methods:

Animals

Adult male Sprague-Dawley rats were used. They were fed a standard diet of food pellets and tap water. Prior to experiments the rats were deprived of food but not water for 48 hours in individual cages with wire mesh bottoms.

Drug and chemicals

Gastrin I 1-17 (15-leucine) was purchased from Fluka AG, Basle, Switzerland, and stored at -20°C. Fresh solutions were prepared each day by dissolving the gastrin in sterile saline (0.9 %) or deionized water (as specified). The biological activity of gastrin-17 (15-leucine) has been ascertained by Dockray et al. (1976). Gastric perfusates were 0.1 M buffers; citrate-HCl for pH 2.5 and citrate-phosphate for pH 4.0, 5.5 and 7.0.

Perfusion and infusion

Under chloralose anesthesia (1.2 ml 5 % solution i.p.) the abdomen was opened through a midline incision. A flanged polyethylene catheter (Portex Ltd, Hythe, England) 2 mm in diameter, was inserted through the rumen and secured with a

purse string suture. An intravenous cannula (nr 10, Portex Ltd) was inserted into the external jugular vein. Constant rate Harvard infusion pumps were used. The rate of intravenous infusion was 2 ml/h. All animals were perfused and infused for 2 hours and the appropriate amount of chloralose was added to the abdominal cavity to maintain anesthesia. Two sets of experiments were performed:

1/ The effects of luminal gastrin were examined using a gastric perfusion rate of 3.6 ml/h and allowing the stomach to drain through the intact duodenum. No visible gastric distension was observed. One group of rats received a saline infusion i.v. and a gastric perfusion with deionized water. Water was chosen since saline may interfere with gastrin binding (Fiddian-Green et al., 1978). A second group of rats received an i.v. infusion of gastrin dissolved in saline (10 µg/kg/h) and a gastric perfusion with water. A third group received saline i.v. and gastrin dissolved in water (10 µg/kg/h) intragastrically. The pH of the gastric perfusate was 6.2.

2/ The effects of intragastric pH on histidine decarboxylase activity were examined using a gastric perfusion rate of 16 ml/h. The duodenum was transected and the proximal part brought out through the midline incision for drainage. Four groups of rats were perfused with

buffers of the following pH's: 2.5, 4.0, 5.5 and 7.0. These treatments do not affect serum concentrations of endogenous gastrin (unpublished observation). Half the rats in each group received saline i.v., the other half (10 µg/kg/h) i.v.

Determination of histidine decarboxylase activity

At the end of two hours, rats were killed by exsanguination, the stomachs were removed, opened along the greater curvature and rinsed in ice-cold saline. The mucosa of the oxyntic gland area was scraped off and homogenized in ice-cold 0.1 M phosphate buffer, pH 6.9 - 7.0, to a final concentration of 100 mg (wet weight)/ml. Aliquots (0.5 ml) of the homogenate were incubated with 4×10^{-4} M 1-^{14}C-L-histidine (1.3 mC/mmole; New England Nuclear) in the presence of 10^{-5} M pyridoxal-5-phosphate and 5×10^{-4} reduced glutathione at +37°C for one hour under nitrogen. Total reaction volume was 0.53 ml. The enzyme reaction was stopped by acidification and the amount of $^{14}CO_2$ produced was determined as described in detail elsewhere (Håkanson & Rönnberg, 1974; Håkanson et al., 1977). Enzyme activities are expressed as pmoles $^{14}CO_2$ produced per mg tissue (wet weight)/h.

Statistical analysis

Comparisons between groups were made using Student's *t*-test for unpaired observations or analysis of variance (where appropriate). Differences with a P value of less than 0.05 were considered significant.

Results:

Effects of luminal gastrin

Three groups of rats were examined. Control rats, receiving no gastrin, had a low histidine decarboxylase activity (7.9 $\pm$ 1.7). The second group of rats, receiving intravenous gastrin, displayed a marked activation of histidine decarboxylase (20.8 $\pm$ 3.4; $P < 0.005$). The third group of rats, receiving the same dose of gastrin but in the gastric perfusate, displayed no activation of histidine decarboxylase (5.9 $\pm$ 1.0). The data are summarized in Fig. 1.

Effects of intragastric pH on gastrin-induced histidine decarboxylase activation

When the i.v. infusion was saline, buffer perfusion of the stomach did not activate histidine decarboxylase over anesthetized control rats within the pH range studied. When

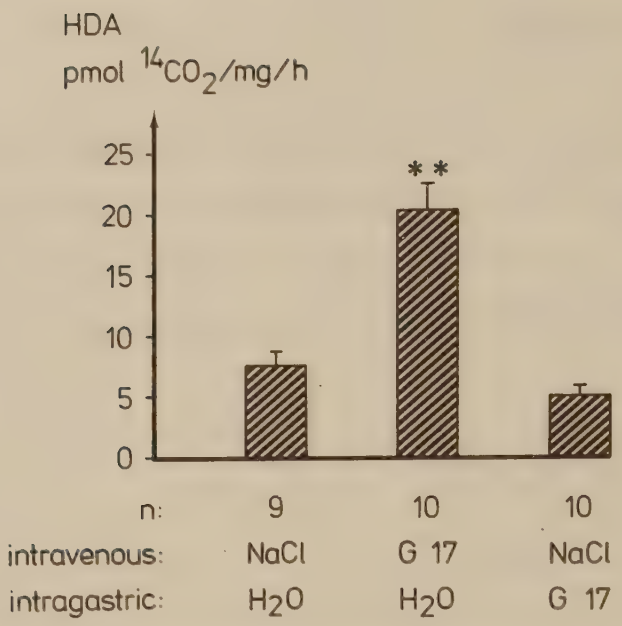

*Fig. 1 Histidine decarboxylase activity (HDA) in rats with gastric perfusion and intravenous infusion. Mean ± S.E.M. ** denotes P < 0.005 compared with controls (no gastrin-17). The number of rats in each group is indicated.*

gastrin was infused intravenously, histidine decarboxylase was markedly activated (P < 0.01; Student's t-test) over saline-infused animals with the same intragastric pH. The gastrin-induced activation was of the same magnitude throughout the pH range studied (analysis of variance). The data are summarized in Fig. 2.

Discussion:

Data are accumulating that upon stimulation of the gastrin cells gastrin is released into the lumen of the stomach as

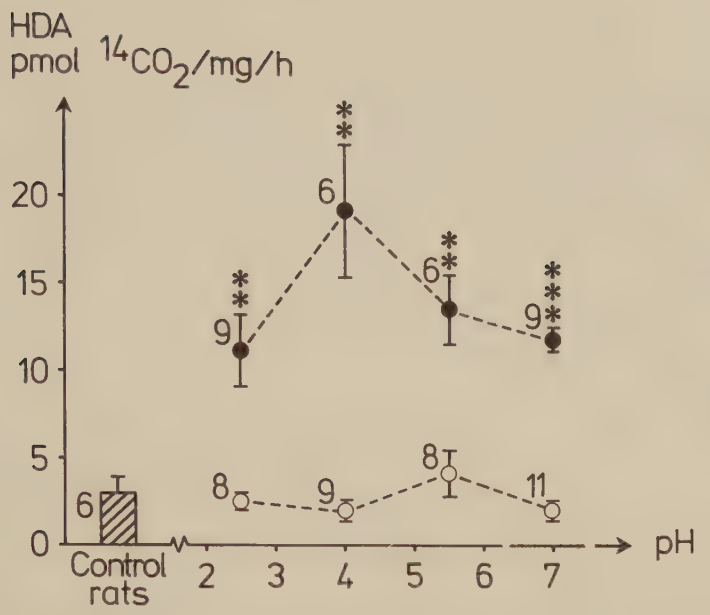

*Fig. 2 Histidine decarboxylase activity (HDA) in four groups of rats receiving gastric perfusion with different pH's (x-axis). Half the rats in each group received i.v. gstrin-17 (closed circles), the other half i.v. saline (open circles). Mean + S.E.M. The number of animals is indicated. Asterisks indicate statistical difference between groups perfused with the same pH (** : P < 0.005; *** : P < 0.001). Control rats received no gastric perfusion.*

well as into the circulation (Jordan & Yip, 1972; Andersson & Nilsson, 1974; Schofield et al., 1976; Fiddian-Green et al., 1978; Vinik, 1978; Uvnäs-Wallensten, 1978). Whether this phenomenon is physiologically significant or not remains to be established. There have been suggestions that luminal gastrin exerts biological actions from within the lumen of the gut (Johnson et al., 1978; Fiddian-Green et al., 1978). As expected, intravenous infusion of a large dose of gastrin-17 raised the histidine decarboxylase activity. Intragastric perfusion with gastrin-17 failed to reproduce this effect. This could mean either that the endocrine cells

containing the enzyme do not respond to gastrin-17 from the lumen or that they are inaccessible to gastrin-17 from the lumen under the conditions of the experiment. However, our failure to reproduce the enzyme-activating effect of intravenous gastrin-17 by luminal gastrin-17 does not mean that also other target cells must be inaccessible or non-responsive to luminal gastrin.

One way of stimulating the gastrin cell is by elevating the intragastric pH (Håkanson & Liedberg, 1970; Becker et al., 1973; Håkanson et al., 1974, 1975; Walsh et al., 1975). Surgical procedures and pharmacological agents capable of elevating the intragastric pH activate histidine decarboxylase (Håkanson et al., 1975). Since gastrin regulates the state of activity of the enzyme (Håkanson et al., 1974), the possibility exists that the sensitivity of the histamine cell to gastrin stimulation is dependent on the intragastric pH. In the present study we were unable to demonstrate such an effect.

This study was supported by the Swedish Medical Research Council (04X-1007, 14X-4144) and by the Albert Påhlsson Foundation.

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MECHANISMS OF GASTRIC ACID SECRETION AFTER PYLORUS AND OESOPHAGUS LIGATION IN THE RAT

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SUMMARY

1. The effect of vagotomy on gastric acid secretion was studied in chronic gastric fistula rats at various times after denervation. In these rats basal and pentagastrin-induced acid output was permanently reduced. Thus, the magnitude of the acid response to pentagastrin in the conscious fistula rat is dependent upon an intact vagus.

2. The acid response to pylorus ligation in vagally intact rats was unaffected by drainage of the stomach and therefore not caused by distension. Bilateral vagotomy, performed simultaneously with the ligation, completely abolished acid secretion, while unilateral vagotomy reduced the acid output by half. Hence, in innervated rats, an intact vagal impulse flow appears to be essential for the acid response to pylorus ligation. When the pylorus ligation was performed 2-8 weeks after truncal vagotomy, the acid output showed a progressive return towards pre-denervation values. In the denervated rats the acid response to pylorus ligation was blocked by drainage of the stomach and therefore probably caused by distension, a mechanism which is independent of the vagal impulse flow.

3. The response to pylorus ligation in innervated rats was blocked by atropine and chlorisondamine but not by metiamide. In the denervated rats, the response to pylorus ligation was blocked by all three drugs.

4. Following ligation of both the pylorus and the oesophagus the acid response was poor. With drainage of the oesophagus the acid response was much enhanced, suggesting that oesophageal distension inhibits acid secretion. In the vagotomized rat the poor acid response to oesophageal + pyloric ligation could not be overcome by drainage of the oesophagus. Distension of the innervated stomach enhanced the acid response. In chronically, but not in acutely vagotomized rats, gastric distension brought about a good acid response suggesting that gastric reflex mechanisms can activate acid secretion through vagal and/or intramural pathways. The stimulatory mechanism initiated by gastric distension can overcome the inhibition induced by oesophageal dilatation. Both in innervated and denervated rats the response to gastric distension was inhibited by atropine, chlorisondamine and metiamide.

5. The results suggest that in the innervated rat vago-vagal reflexes are important for the gastric hypersecretion following ligation of the pylorus, and for the acid response to gastric distension following ligation of the pylorus and oesophagus. In the chronically vagotomized rat local intramural reflexes elicited by gastric distension are responsible for the acid response.

INTRODUCTION

Ligation of the pylorus is a powerful stimulus for acid secretion in the rat. The mechanism behind this reponse is not fully elucidated although available evidence suggest the involvement of a vago-vagal reflex (Brodie, 1966; Brodie & Knapp, 1966). Shay, Komarov & Gruenstein (1949) and Donald & Code (1952) reported that acute vagotomy completely abolished the acid response to pylorus ligation. On the other hand, both Shay et al. (1949) and Donald & Code (1952) found a reduced rather than abolished acid response in vagotomized rats subjected to pylorus ligation 5-6 months after the denervation. The effect of vagotomy on gastric acid secretion in the rat is a matter of some controversy. It seems to depend on the technique used for stimulation of gastric secretion and collection of gastric juice. In rats with gastric pouches, the acid output in response to a variety of stimuli was found to be the same in innervated (Pavlov) and denervated (Heidenhain) preparations (Lundell & Svensson, 1973). In studies on chronic gastric fistula rats it has been found that vagotomy profoundly suppressed basal acid output and prevented the acid secretory response to maximal and supramaximal doses of both pentagastrin and histamine (Lin & Alphin, 1958; Håkanson & Liedberg, 1970, 1971; Kowalewski, 1971). By contrast Lane, Ivy & Ivy (1957) observed no reduction in the basal acid output three weeks after truncal vagotomy. In a recent study on rats with chronic gastric fistulas in the totally bypassed stomach (Håkanson, Hedenbro, Liedberg & Vallgren, 1979), a slight but statistically significant secretory response to pentagastrin or histamine could be elicited after vagotomy. However, in these experiments acid secretion after vagotomy never exceeded 10% of that in control, vagally innervated rats.

The present study deals with the mechanism of gastric acid secretion following pylorus and oesophagus ligation in innervated and vagotomized rats.

MATERIAL AND METHODS

Animals. Male Wistar rats (200-300 g body weight if not otherwise specified) from the same breeder were used. All animals were fasted for 24 hours before secretory studies or surgery.

Drugs. Atropine (ACO Inc., 10 mg/kg) and Peptavlon^{R} (ICI; pentagastrin, 125 or 250 µg/kg) were given subcutaneously. Metiamide (Smith, Kline & French) and chlorisondamine (Ciba-Geigy) were given intraperitoneally, 30 mg/kg and 5 mg/kg,

respectively. In experiments with pylorus-ligated rats drugs were given at the time of ligation. In experiments with fistula rats drugs were given after two hours of collection of gastric juice.

Surgical procedures. All operations were performed under diethyl ether anaesthesia using clean, but not sterile instruments. Vagotomy was performed by dividing both vagal trunks immediately below the diaphragm as described in detail by Shay et al. (1949). In animals intended for long-term studies, a pyloroplasty was always performed at the same time in order to prevent food retention and fatal gastric dilation (Shay et al., 1949). Unless otherwise specified, vagotomized rats were left for 8 weeks before being used in experiments. About three weeks before experiments chronic gastric fistulas modified after Bel, Leurat, Nesmos & Girard (1966) were fitted into the rumen and brought out immediately below the left costal margin. Usually, the fistulas remained in place with no complications for at least one month. Thereafter, the fistulas were rejected in increasing numbers. Before actual rejection there was often evidence of leakage around the fistulas.

Collection of gastric juice. During experimentation, conscious rats with chronic gastric fistulas were restrained in Bollman cages. The stomach was allowed to drain through the opened fistula for one hour, after which basal secretion was collected for 2 hours. Pentagastrin was given s.c. and 4 further one-hour portions collected. Basal acid secretion is defined as the portion collected during the second hour. Pentagastrin-induced secretion is that which is collected during the first hour after pentagastrin injection minus the basal secretion. Pylorus ligation was performed under light ether anaesthesia which does not interfere with the gastric secretory response (Lee & Thompson, 1967). A tight ligature was placed around the pylorus. If oesophagus ligation was also performed, great care was taken not to damage the vagus trunks. The anterior vagus trunk was dissected bluntly and gently retracted to permit an oesophageal ligature to be applied in the diaphragmatic hiatus. At this level the posterior vagal trunk runs separately from the oesophagus. Four hours after the ligation (unless otherwise stated) the animals were again anaesthetized, the stomach removed and its content collected. In one series of experiments the oesophagus was drained through a catheter inserted through the rumen and brought out below the left costal margin. In another series of experiments on pylorus- and oesophagus-ligated rats various volumes of 0.9% saline were injected into the stomach via the rumen. The animals were killed 4 hours later and the gastric acid was titrated.

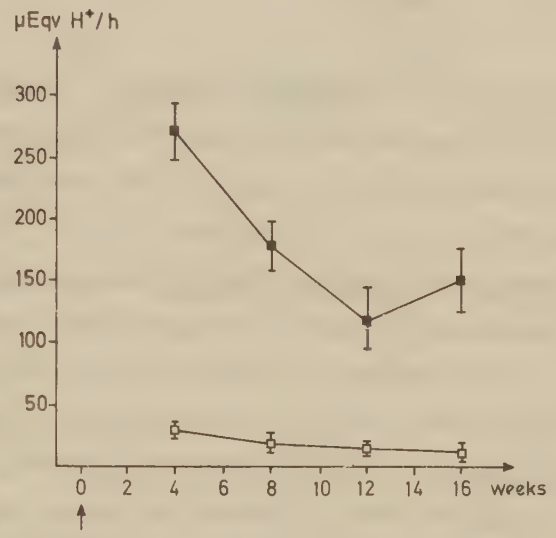

Fig. 1

Gastric acid output after pentagastrin (125 μg/kg s.c.) in chronic gastric fistula rats studied at various times after surgery. Arrow denotes either insertion of gastric cannula alone (filled squares) or insertion of cannula plus bilateral truncal vagotomy and pyloroplasty (open squares). Eight to ten animals in each group. Mean ± S.E. of the mean. The weight of the rats at the start of the study was 200-270 g for the innervated group and 150-220 for the denervated one. At the end of the study the innervated rats weighed 300-370 g and the denervated ones 200-300 g. Leakage was increasingly observed 1-2 months after insertion of the fistula and may have contributed to the progressively reduced acid output.

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All animals received 10 ml 0.9% saline subcutaneously prior to the secretory studies in order to prevent dehydration. Acid output was determined by titration with 0.02 N NaOH, using phenolphthalein as indicator.

RESULTS

1. Gastric acid secretion in chronic gastric fistula rats. Pentagastrin-induced secretion (Fig. 1) as well as basal acid output (not shown in figure) were markedly suppressed in the vagotomized rats. This suppression was not reversed with time (16 weeks after vagotomy).
2. Gastric acid secretion in pylorus-ligated rats. The maximum volume and acid output was found after 5-6 hours (Fig. 2). Subsequently, accumulation of gastric juice ceased; actually, both volume and acid content diminished 12-20 hours after ligation. Pylorus ligation alone was a strong stimulus for gastric acid secretion, and no further stimulation could be elicited by pentagastrin (250 µg/kg) injected at the time of ligation (Fig. 3). Dividing the anterior or posterior vagal trunk at the time of ligation lowered the acid output approximately by half. Sectioning of both vagal trunks inhibited acid secretion almost completely (Fig. 3). In one study pylorus ligation was performed on rats which had been vagotomized 2, 4, 6 or 8 weeks previously. Results are shown in Fig. 4. By prolonging the time interval between denervation and pylorus ligation a progressive increase in the acid response to pylorus ligation was observed.

   In innervated rats, the acid response to pylorus ligation was greatly reduced by atropine and chlorisondamine but not by metiamide (Fig. 5). In chronically denervated rats all three drugs inhibited the acid response (Fig. 5). In one experiment 14 gastric fistula rats were pylorus-ligated and after regaining consciousness restrained in Bollman-type cages. One group had the fistula open while the other had it closed. The acid that was collected from the open fistula during 4 hours was 664 µEqv $\pm$ 98 (S.E. of mean, n = 7). The acid that accumulated in the stomachs of the latter group during the same time was 744 µEqv $\pm$ 180 (S.E. of mean, n = 7). Subsequently, the same experiment was repeated using 12 rats which had been vagally denervated 8 weeks before the pylorus ligation. In the group with open fistula (body weight 170-230 g) the acid output was 18 µEqv $\pm$ 4 (n = 6); the corresponding value in the group with closed fistula (body weight 200-270 g) was 178 µEqv $\pm$ 42 (n = 8) ($p < 0.001$).

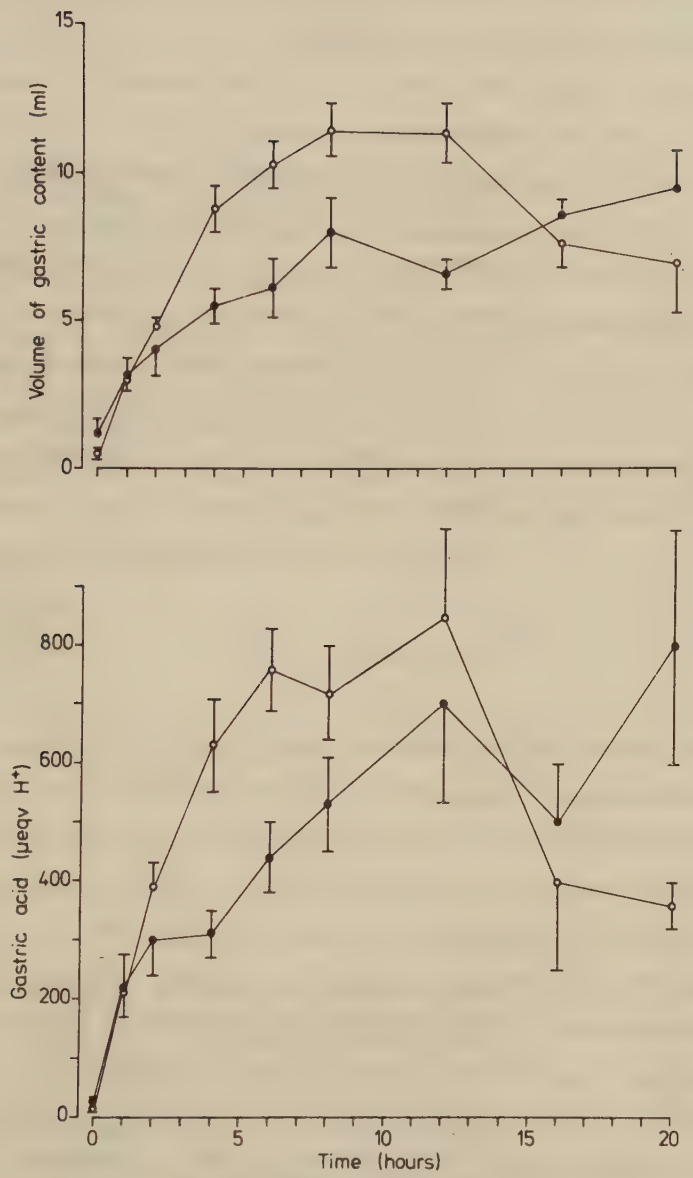

Fig. 2

Volume and amount of acid in the gastric content at various time intervals following pylorus ligation of innervated (○) and chronically vagotomized (●) rats. Denervation was made approx. 2 months before the experiment. Eight to twenty-three animals in each group. Mean ± S.E. of the mean.

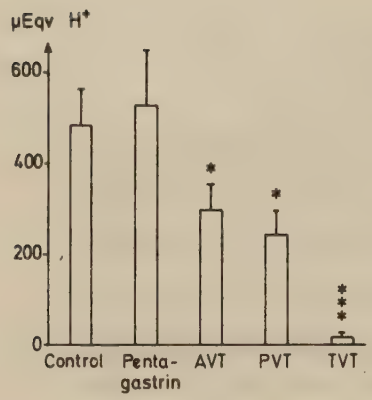

Fig. 3

Gastric acid output after pylorus ligation for 4 hours. Control rats received no further treatment. Pentagastrin = injection of pentagastrin, 250 μg/kg at the time of ligation, AVT = section of anterior vagal trunk, PVT = section of posterior vagal trunk, TVT = total (bilateral) vagotomy. Four animals in each group, body weight range 160-180 g. Mean ± S.E. of the mean. * for $0.01 < p < 0.05$, *** for $p < 0.001$.

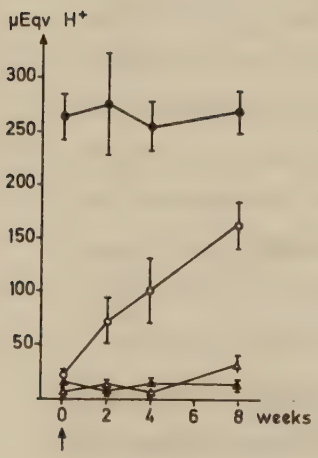

Fig. 4

Gastric acid output in pylorus-ligated rats at different times after sham operation by laparotomy alone (closed symbols) or truncal vagotomy with pyloroplasty (open symbols). Circles denote pylorus ligation alone, triangles pylorus plus oesophagus ligation. Four animals in each group, body weight range 150-300 g. Mean ± S.E. of the mean.

3. Gastric acid secretion following ligation of the pylorus and of the oesophagus.

After pylorus ligation in combination with oesophagus ligation the acid response was poor. This was so also at various times after vagotomy (Fig. 4). Instillation of 0.9% saline into the stomach of innervated rats evoked copious acid secretion (Fig. 6 and 7). The maximum response was after instillation of 12 ml (Fig. 6). Acute vagotomy prevented the acid response to distension (Fig. 7). However, 8 weeks after vagotomy the response to instillation of saline had returned. Now the maximum response was after 9 ml (Fig. 6). Treatment with atropine, metiamide or chlorisondamine blocked the acid response to distension in both innervated and vagally denervated rats (Fig. 7).

In innervated rats, drainage of the ligated oesophagus caused a return of the acid response to pylorus ligation (Fig. 8). In acutely or chronically vagotomized rats drainage of the oesophagus had no such effect.

DISCUSSION

Pentagastrin and pylorus ligation evidently stimulate gastric acid secretion in the rat by entirely different mechanisms. Pentagastrin probably has a direct effect on the parietal cells although it seems to depend upon an intact vagal innervation of the parietal cells in order to be fully effective. In the absence of vagal innervation the acid secretory response to pentagastrin in chronic gastric fistula rats was poor. The reduced response to pentagastrin following vagotomy appeared to be permanent (4 months studied), indicating that during this time no reinnervation occurred. Earlier studies have indicated that the vagus is important for the acid response also to pylorus ligation (Harkins, 1947; Shay et al., 1949; Alexander & Merendino, 1952; Donald & Code, 1952; Rivilis, Yaffe & Preshaw, 1968). However, the suppression of acid secretion after vagotomy was found to be transient and almost fully reversible.

The mechanism by which pylorus ligation stimulates acid secretion is not clear. Acid secretion was stimulated by pylorus ligation also when the stomach was continuously drained via a gastric fistula and hence, distension of the stomach does not seem to be important. Conceivably, receptors in the pyloric part of the stomach wall are activated by the ligature itself (cf. Brodie, 1966). Pylorus ligation seems

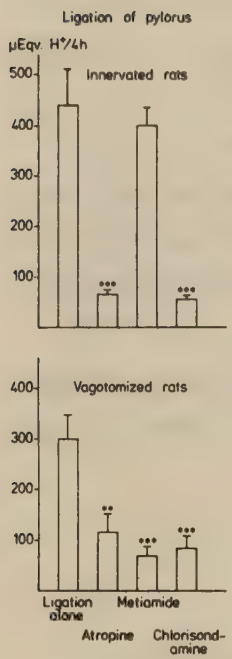

Fig. 5

Effects of atropine, metiamide or chlorisondamine on the acid response to pylorus ligation. Upper panel shows the results with innervated rats, lower panel those with chronically vagotomized rats. Six animals in each group, body weight range 180-220 g. Mean ± S.E. of the mean. ** $0.001 < p < 0.001$, *** $p < 0.001$.

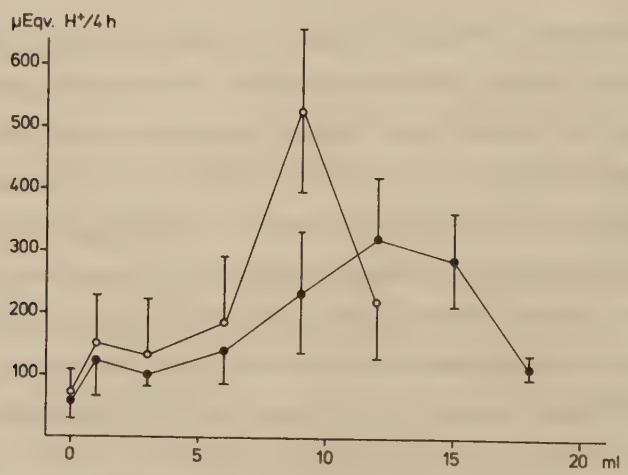

Fig. 6

Acid response to distension of the stomach with increasing volumes of 0.9% saline. The rats subjected to ligation of both the pylorus and the oesophagus were killed 4 hours after the injection of saline. Closed circles, innervated animals; open circles, chronically vagotomized animals. Three to six animals in each group. Mean ± S.E. of the mean.

to cause maximal stimulation, since acid secretion was not further increased by injection of pentagastrin. Atropine and ganglionic blocking agents (chlorisondamine) but not histamine H_2-receptor antagonists (metiamide) lowered the acid response (see also Dai, Ogle & Lo, 1975; Okabe, Takeuchi, Urushidani & Takagi, 1977). On the whole, our results agree with those of Brodie (1966) and Brodie & Knapp (1966) who suggested the involvement of a long reflex, resumably vago-vagal, in the acid response to pylorus ligation. In the present study we also confirmed the finding that acute vagotomy greatly reduced the acid response to pylorus ligation (see Brodie, 1966). However, with time after vagal denervation the response gradually returned almost to pre-denervation values. The return of the response in chronically vagotomized rats probably reflects the gradual enhancement of a local reflex arc, conceivably activated by distension, since it was prevented by drainage of the stomach. The gradual return of the acid response to pylorus ligation following denervation may suggest that with time the vagal "tone" on the parietal cells is replaced by short, intra-mural reflexes activated by gastric distension. This hypothesis is compatible with the finding that oesophageal ligation, which prevents gastric distension by saliva, inhibited the secretory response to pylorus ligation. In innervated rats this inhibition probably reflects activation of an inhibitory vagal reflex arc (cf. Brodie & Knapp, 1966; see also Sjödin, 1975), elicited by the back-up of saliva in the oesophagus; if the oesophagus is drained the acid response returns. In the denervated rats the inhibited acid response probably reflects lack of distension since saliva no longer reaches the stomach. Distension induced by instillation of 0.9% saline into the stomach of rats subjected to ligation of both the pylorus and the oesophagus evoked copious acid secretion in both chronically vagotomized and innervated rats but not acutely vagotomized animals.

The mechanism of inhibition of acid secretion following oesophageal ligation in innervated rats was interpreted by Brodie & Knapp (1966) as evidence of a reflex inhibition of central vagal nuclei, brought about by the back-up of saliva in the mouth and pharynx, which stimulated vagal afferent nerves. The stimulation of secretion by insulin and 2-deoxyglucose, both of which are known to stimulate vagal nuclei, were means of overcoming the proposed afferent inhibition of the vagus. These observations were interpreted as evidence for an inhibitory vagal reflex arch (Brodie & Knapp, 1966). Levine (1965) postulated that the gastric inhibition was due to the prevention of

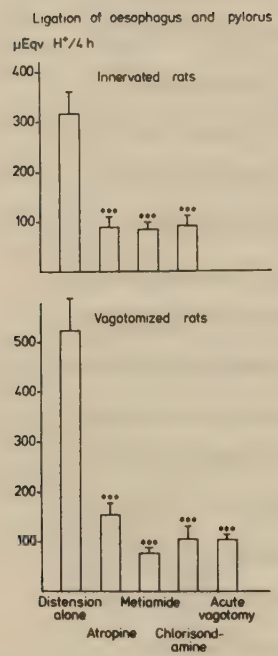

Fig. 7

Effects of atropine, metiamide or chlorisondamine on the acid response to gastric distension in rats subjected to pylorus and oesophagus ligation. Upper panel shows the results with innervated rats, lower panel those with chronically and acutely vagotomized rats. Six animals in each group. Mean ± S.E. of the mean. *** $p < 0.001$.

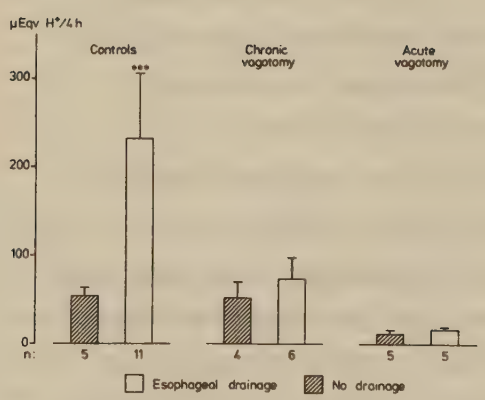

Fig. 8

Effect of drainage of the ligated oesophagus on the acid response to pylorus ligation. Left, innervated rats; middle, chronically vagotomized rats; right, acutely vagotomized rats. n = number of animals. Mean ± S.E. of the mean. *** $p < 0.001$.

saliva, which he considered to contain a potent gastric acid stimulant, from reaching the stomach. He also reported that removal of the salivary glands greatly reduced gastric secretion in the pylorus-ligated rat and that administration of saliva to oesophagus-ligated rats stimulated gastric secretion. Conceivably, therefore, saliva may be one factor behind the secretory response to pylorus ligation.

To summarize: In the chronic gastric fistula rat the acid response to pentagastrin seems to depend upon a direct vagal tone to sensitize the parietal cells. After dividing the vagi, no matter how long after the denervation, the parietal cells responded poorly to pentagastrin. Our results suggest that the acid response to pylorus ligation of innervated rats results from activation of a vago-vagal reflex arc by the ligature itself. In chronically vagotomized rats the gradual return of the capacity to respond to pylorus ligation with acid secretion reflects the gradual take-over of an intramural reflex arc, presumably activated by the distension caused by swallowed saliva. Oesophageal ligation results in two things. First, accumulated saliva causes oesophageal distension, second, saliva is prevented from causing gastric distension. In innervated rats drainage of the oesophagus caused a return of the acid response to pylorus ligation. Hence, in innervated rats oeseophageal dilatation counteracts the acid response to pylorus ligation. Gastric distension is a potent stimulus for acid secretion following ligation of the pylorus and oesophagus both in innervated and in chronically vagotomized rats. In innervated rats the response seems to be elicited by a vago-vagal reflex since acute vagotomy prevents it. In chronically vagotomized rats the response to gastric distension has returned; the results of the present study suggest that now local reflex mechanisms within the gastric wall play an important role.

No attempt was made in this study to identify the chemical signals responsible for exciting the parietal cells. Nonetheless, the results suggest that histamine is not involved in the secretory response to pylorus ligation of innervated rats since the acid response was not blocked by histamine H_2-receptor antagonists. In all the other experimental models tested the acid response was blocked by the H_2-blocker used. In addition, the acid response in all the various experimental models were blocked by atropine and a ganglionic blocking agent. The results therefore suggest an interplay of several factors, presumably neurogenic.

ACKNOWLEDGEMENTS

Grant support from the Swedish Medical Research Council (04X-1007), the Påhlsson Foundation, and the Medical Faculty of Lund, Sweden.

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1980